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STUDIES ON THE SPINAL CORD AND MEDULLA OF CYCLOSTOMES WITH SPECIAL REFERENCE TO THE FORMATION AND EXPANSION OF THE ROOF PLATE AND THE FLATTENING OF THE SPINAL CORD[1]

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EIGHTY-SEVEN FIGURES

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INTRODUCTION

This paper has grown out of the study of the caudal heart and the spinal cord and nerves related to it in Polistotrema. Parts of that study which are not yet completed will be published later and will deal with the origin, distribution, and phylogeny of the spinal nerves; the origin of muscle sense organs in connection with the specialized muscles of the caudal heart; and

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certain ganglion cells, possibly sympathetic, which are found along the course of the spinal and vagus nerves. The present paper includes a study of the mode of origin of the fourth ventricle in several groups, an account of a structure similar to the fourth ventricle found in the caudal part of the spinal cord of an adult Polistotrema, and a study of the development of the central canal, and the causes underlying the flattening and the ventral indenting of the spinal cord in Cyclostomes.

Material and its preparation

Since the material for this paper came from such diverse sources, and such different modes of technique were employed, too much time and space would be consumed if a detailed description were given of each method employed. With the exception of the Petromyzon material, which was fixed in Flemming, corrosive-acetic, and Perenyi's fluid, my own material was fixed either in Tellyesnicky's or Bouin's fluid. It was sectioned after paraffin or the combined celloidin-paraffin method of imbedding. The latter method gave by far the better results, since it appears to have all of the advantages of celloidin in causing almost no shrinkage and its ability to hold yolk granules and blood corpuscles intact; besides allowing the sections to be cut as thin as paraffin alone, and causing no more difficulty in manipulation. For the most part the sections were stained in Heidenhain's iron hæmatoxylin and counterstained in an alcoholic solution of orange G plus a little acid fuchsin. In a few instances, in very young Petromyzon embryos, where all the tissues were filled with yolk, carmine and hæmalum were used to advantage.

Acknowledgments are due to Prof. T. G. Lee for the gift of a very complete series of Petromyzon embryos, which were obtained from the Connecticut River. Also to Prof. R. E. Scammon for the loan of his very complete serial collection of Squalus embryos, and to Prof. J. B. Johnston for the use of a similar serial collection of Amblystoma embryos. The splendid series of human and pig embryos belonging to the Institute of

Anatomy of which Prof. C. M. Jackson is director, were especially valuable.

It is a great pleasure to the writer to have this opportunity of expressing his obligations to Prof. J. B. Johnston for his valuable suggestions and friendly criticism of this work.

A model of the caudal end of the spinal cord from a region a little in front of the caudal hearts to its extreme tip was prepared in four sections from the 20 cm. Polistotrema series. Also a model in two pieces was prepared of the cavity of the so-called first roof plate expansion and the enlarged central canal of the above mentioned series of Polistotrema. These models were constructed out of blotting paper after a modification of the Born method. Tracings of each section were made on ordinary writing paper with the aid of an Edinger-Leitz drawing apparatus, using a magnification of 100 diameters, and afterward each tracing was carefully checked up for accuracy with a higher magnification. At the very outset two base or projection lines were drawn on the first tracing, one following the median longitudinal plane, and the other a horizontal line drawn at right angles to the first line, passing along the ventral border of the notochord. These projection lines were added by pencil to all of the succeeding tracings after the following manner: The second tracing was carefully fitted over the first, after placing both over a plate glass covered box, containing an electric light reflected upward, and in like manner these lines were added to the third tracing from the second, and so on to the end of the series. Then with the aid of carbon paper these tracings and projection lines were transferred to sheets of blotting paper, having a definite thickness, previously determined after the following method. When thoroughly cooled after an immersion in melted paraffin, the blotting paper selected should have a thickness, equal to the thickness of the section multiplied by the magnification used, which in this case totalled 1.5 mm. The sheets of blotting paper containing the transposed tracings were then immersed in melted paraffin, drained and cooled. After which the outlines of the tracings were cut out with a sharp scalpel, and the sections were built up in regular order. In

part to maintain this regular order and in part to add strength to the model, a copper wire was inserted through each section, passing through the point of intersection of the two projection lines. After a certain number of sections had been strung on this wire they were securely fastened to each other by pins.

HEREDITARY AND MECHANICAL CAUSES UNDERLYING THE FORMATION OF THE FOURTH VENTRICLE AND THE TELA CHORIOIDEA

To the writer this problem appears to be primarily phylogenetic rather than ontogenetic; consequently this study begins with the lowest vertebrates, and is approached as a problem of evolution.

1. Roof plate of the medulla oblongata

A. Amphioxus. Unfortunately I have not had access to any embryological Amphioxus material so that my inferences will have to be drawn entirely from adult material.[2] At the outset it can be maintained with a considerable degree of safety that the adult Amphioxus brain contains nothing which can be homologized with the fourth ventricle of higher vertebrates. Figure 67, which pasess through the highest point of the anterior ventricle ($V._1$) demonstrates clearly that there is nothing here comparable with the fourth ventricle. For the dorsal portion of the cavity is much narrower than the ventral portion, and there is absolutely nothing in the way of a thin and expanded roof plate. In fact, the dorsal portion of the cavity is fairly filled up with processes from ependymal cells.

A few sections behind the first ventricle there appears a small isolated cavity in the roof plate region of this series, designated as the posterior ventricle (fig. 68, $V._2$), and a considerable distance behind this cavity, there is a second dorsal cavity, also designated as the posterior ventricle (fig. 69, $V._2$). This is somewhat larger than the previous cavity, and can be readily located

[2] In order to eliminate minor details of description from the text, very complete and detailed descriptions of the figures have been given at the end of the paper, to which the reader's attention is directed.

from its position immediately above a dorsal group of large nerve cells (*M'.C'.*). From the figures by Hatschek, Willey, Sterzi, and others it is evident that these isolated roof cavities described above, were once a part of a common central cavity of the embryonic brain, which later in development, became isolated through an invasion of ependyma, and it is entirely possible that this region of the central cavity in the embryo was much more suggestive of the fourth ventricle. Judging from the adult alone they may be looked upon merely as vestiges of the embryonic central canal.

B. Polistotrema (*Bdellostoma*) My embryonic material of Polistotrema confirms the statements of Price, von Kupffer, and Dean that Polistotrema possesses well-developed ventricles in the embryo; the expansion being fully as great as in a similar stage of Petromyzon. As development proceeds the lateral plates increase in thickness from additions of fibers and cells until the fourth ventricle becomes reduced to a canal, but little larger than the central canal of the spinal cord.

Sanders, Holm, Miss Worthington, Sterzi, Cole, and Nicholls describe and figure the fourth ventricle about as it is shown in figure 63. There is some little discrepancy in the terminology used, due largely to the differences of opinion as to whether or not Polistotrema has a cerebellum. If the posterior lobes of the mesencephalon should in the light of future investigation turn out to be a cerebellum, then the boundaries of the metencephalon will have to be carried further forward than we have indicated, and the so-called sinus mesocœlicus of Nicholls (*S.M.*) will have to be called the anterior dilation of the fourth ventricle of Miss Worthington's description.

My transverse series through the brain of Polistotrema show the condition of the ventricles to be almost identically the same as Sterzi and Nicholls found them. Until the embryological and functional areas of the brain have been better worked out it seems advisable to the writer to let the posterior border of Nicholls' sinus mesocœlicus (fig. 63, *S.M.*) mark the boundry line between the mesencephalon and the metencephalon, and to regard his isthmic and ventricular canals (figs. 63 and 65, $A._4V.$)

as dorsal and ventral portions of the anterior end of the fourth ventricle. For the reason that in Polistotrema they extend some distance behind the posterior tip of the mesencephalon, and the dorsal or posterior canal is shown in transverse section (fig. 65) to lie close to the dorsal surface, which is then the only part of the fourth ventricle to retain the characteristic dorsal position of the higher vertebrates. These two canals appear in this series about as Nicholls has described them, the dorsal (isthmic) is the larger and contains Reissner's fiber. Although probably subject to a considerable variation, these canals apparently extend further caudad in this series than Nicholls represents them. Also in this series the dorsal canal (isthmic) gives off one or two branches at the level of the posterior tip of the mesencephalon (cerebellum of Miss Worthington), which run parallel to the main canal, but a little to one side and below (fig. 65, *A.$_4$V.*). After travelling side by side for some little distance in a mass of spongy ependymal tissue close to the roof plate, they reunite in the dorsal canal, and soon afterward both the dorsal and ventral (isthmic and ventricular) canals unite in a common canal, which is little if any larger than the central canal of the spinal cord. This constricted portion of the fourth ventricle (fig. 63) continues caudad, rather deep-seated in a mass of loose vascular ependyma, until the posterior end of the medulla is reached, where it expands into a much larger vesicle or sinus, designated as the posterior dilation of the fourth ventricle (figs. 63 and 66, *P.$_4$V*). Behind this it soon tapers down into the ordinary central canal of the spinal cord.

A glance at figures 63 to 66 suffices to show that the fourth ventricle of Polistotrema is greatly reduced as compared with that of Petromyzon. This is due probably to the rapid increase of fibers and cells in the lateral plates. Notwithstanding this reduction in size and general alteration in appearance and structure, the walls of the fourth ventricle in Polistotrema, although representing a greatly modified chorioid plexus, are unquestionably capable of producing cerebro-spinal fluid. The posterior dilation of the fourth ventricle (fig. 66, *P.$_4$V.*) contains cerebro-spinal fluid (*S.C.F.*) in the form of a deeply staining feltwork,

which is not ependymal cilia or a tangled Reissner's fiber. Also throughout its entire length, as was noted by Sterzi, the fourth ventricle is enveloped by a rather thick layer of spongy and very vascular ependyma, which would be distinctly favorable for infiltration and possibly for secretion into the ventricle. This rich blood supply is from the blood vessels and sinuses traversing the meningeal membranes, and especially from two large arteries (A. rhombencephalica of Sterzi), one of which appears in figure 64 (*M.A.*). Also it would be quite possible in the anterior part of the fourth ($A._4V.$) for the various canals, which run close to the dorsal, to receive infiltration direct from the outer meningeal blood and lymph sinuses.

Coagulated cerebro-spinal fluid is also to be seen in reduced amounts in the mesencephalic ventricles designated as the posterior mesocœle and the sinus mesocœlicus. They are also surrounded by a layer of vascular ependyma, which, while much thinner than the corresponding layer of the fourth ventricle, doubtless functions as a cerebro-spinal fluid forming organ.

A careful examination of this peculiar modification of the chorioid plexus of the fourth ventricle in Polistotrema leads one to believe that this is not as efficient an organ for the production of cerebro-spinal fluid as the more expanded tela chorioidea of Petromyzon and higher vertebrates.

C. Petromyzon. Petromyzon is apparently the best type that could be selected for obtaining definite information concerning the early history of the formation of the fourth ventricle and its expanded roof plate. 1) It is the lowest living vertebrate that possesses a well-developed fourth ventricle and expanded tela chorioidea in the adult. 2) At no times does the medulla have a pontine flexure. 3) Its central nervous system remains a solid cord of ectoderm until after the cranial and spinal ganglia are well-differentiated. For these and other reasons the early history of the fourth ventricle in Petromyzon has been studied in the effort to determine the essential factors involved in its appearance and growth.

In order to arrive at the fundamental factors involved in the anlage and development of the fourth ventricle in Petromyzon

it is necessary to go back in the ontogeny of the central nervous system to the time when it was a solid cord. Of my embryos which represent stages killed at 5, 7, 9, 10, 11, 12, 14, 16, 18, 20, and 26 days after fertilization, all of the 5 day series revealed the central nervous system as a solid cord or keel of ectoderm cells, formed according to Calberla by a process of delamination. In places neural crest cells can apparently be seen budding off from the dorso-lateral surface of the brain and spinal cord almost identically as described and figured by von Kupffer ('90). It is obvious that the preponderance of yolk granules in all tissues makes accurate observation in these stages very difficult. The majority of the 7 day embryos disclosed a central canal either formed or in the process of formation. Out of a great number of series of 10 day embryos, three were found in which the opening of the central canal had been considerably retarded. In one series the central nervous system was still a solid cord of cells, and in the other two the central canal was in the early stages of formation. These series were selected in preference to similar stages of 7 day embryos for the reason that less yolk was present to obscure the various structures.

To facilitate comparison of similar sections of successive stages, figures 33 to 38 and figures 40 to 53 have been so arranged as to bring several regions of series in a horizontal row, while successive stages of the same region are placed in a vertical row. The conditions at the level of the trigeminal, auditory and vagal ganglia are thus readily compared.

In figure 32 we have a section through the medulla in the region of the VIII ganglion, which portrays very well an extremely early stage in the formation of the central canal. Here the medulla will be seen to be composed of a mass of nuclei imbedded in a syncytium of protoplasm. Excepting in the roof and floor plates, the nuclei have migrated some little distance to either side of the median sagittal plane, presenting the appearance of a rather broad light line surrounded by nuclei. The center of this light streak of protoplasm discloses a very conspicuous furrow or seam (*C.C.S.*), which appears in every section of the central nervous system of this series, very much as it looks in

this section. This furrow or seam represents the position of the future central canal. The protoplasm adjacent to this seam is sufficiently granular to suggest a secretory function at this early stage.

Figures 33 to 35 also taken from a 10 day Petromyzon series exhibit a slightly later stage in the formation of the central canal. Conspicuous cavities (*C.C.*) have appeared in the dorsal and ventral portions of this furrow, which are not only visible throughout the entire medulla, but are continuous throughout the spinal cord. It should be recorded that a few sections through the medulla possessed slightly larger dorsal and ventral cavities than were represented by these figures, but in no case had they approached each other close enough to unite. In other respects, excepting possibly for a few more fibers in the marginal layer, the structure of the medulla has remained about the same as in the previous series. Emphasis should be made of the fact that each of these cavities, when examined with a higher magnification, reveals a certain amount of fine granules, which in some cases may have completely filled the cavity, while in others they are confined to the outer edge, leaving a clear space in the center. The presence of these granules here suggests two processes: 1) disintegration of the central protoplasm, and 2) products of secretion. The lateral migration of the nuclei could be utilized to support either inference. That such a migration of nuclei would be favorable for disintegration is self evident, and secretory cells are usually characterized by having their nuclei somewhat remote from their lumina.

In the 11 day series (figs. 36 to 38) we find that the two isolated dorsal and ventral cavities of an earlier stage have not only united and formed a cleft-like cavity, which may now be designated as the typical embryonic central canal, but that the original dorsal and ventral cavities of this canal, especially the dorsal, have increased notably in size. The narrow central portion (figs. 37 and 38) indicates that this is the place where the protoplasm was last to separate. It should not be confused with a similar condition that occurs later, when the central and ventral portions of the lateral plates migrate inward, fuse, and

completely obliterate that portion of the embryonic central canal. Aside from the change in the central canal the size of the medulla has increased in all directions, but especially laterally, due doubtless to an increase in the number of nerve fibers and cells in the lateral plates. Both roof and floor plates are very thin, comprised of about one layer of nuclei each. The ventral plate may be slightly thicker, due to the addition of a few nerve fibers to the outer layer. Absolutely no stretching of the roof plate has occurred, indicating that it is not under any marked internal pressure. In figure 37 (*B.V.*) branches of the intersegmental blood vessels have stretched out toward the roof of the brain, but at this stage they are too remote from the roof plate to be very active in infiltration. Figure 39 makes clear that the spinal cord has made equal progress with the medulla in developing a cleft-like or typical embryonic central canal. In the cord the dorsal and ventral enlargements of the central canal are shown to be of about equal size.

An additional day (figs. 40 and 41) discloses considerable expansion of the central canal throughout, with the exception of the extreme ventral portion. Very noticeable is the increase in size of the dorsal portion, the future fourth ventricle (cavità della tela coroidea of Sterzi). The roof plate exhibits no signs of stretching. Up to this stage the expansion appears to be due to the migration of the ependymal cells upward and outward, rather than to pressure within. The floor plate will be seen to have increased considerably in thickness through an addition of fibers to its marginal layer, which would obviously tend to make the floor plate more resistant than the roof plate to pressure from within from this time on. Both lateral plates disclose a remarkable growth in thickness due to an addition of both fibers and cells, some of which, however, must be attributed to the fact that the head was sectioned somewhat obliquely (note in fig. 40 that the V and VIII ganglia appear in the same section). But little progress has occurred in the development of the intersegmental blood vessels, so that Sterzi's conjecture, that the embryonic cerebro-spinal fluid does not differ in any way from the ordinary intercellular fluids, would probably hold, if it could be

established that the central protoplasm in disintegration contributed nothing to it. The spinal cord was found to be in about the same condition as the 11 day series.

Moreover an 18 day series (figs. 42 to 44), some 6 days older than the last stage compared, shows practically no change in the shape and size of the central canal or expansion of the roof plate, despite the fact that a notable increase of fibers had occurred in the ventral and median portions of the lateral plates. It should also be recorded that the intermediate stages, as represented by the 14 and 16 day series, exhibited a like state of the central canal. A further interesting condition is revealed from a section of the spinal cord of this series (fig. 45). Here the lateral plates have expanded to such an extent from the formation of fibers in the marginal layer, that the inner surfaces of their central areas have nearly formed a complete concrescence at the center of the original central canal. In other words, the inner surfaces of the lateral plates have met, and are about to fuse, leaving dorsal and ventral cavities (*C.C.*). The ventral one of these cavities will persist as a permanent central canal. In Petromyzon this partial closure of the original embryonic central canal of the spinal cord may have considerable bearing on the increase of internal pressure of cerebro-spinal fluid on the walls of the medulla, during this and later stages. We may regard the period from about the 12th to about the 18th day as a period of rest in the formation of the fourth ventricle in Petromyzon. During this time the processes which would tend to expand the central canal have been met with equal counter forces, which would make for closing it up.

Transverse sections through the medulla of a 20 day Petromyzon series (figs. 46 to 48) demonstrate a marked change in the medulla and its central cavity, which cavity has now assumed the form of a fourth ventricle. A marked increase has taken place in the number of fibers in the ventral plate and in the ventral portion of the lateral plates. This together with pressure from the growing auditory vesicles (*Aud.V.*) and the notochord, has produced a nearly complete concrescence of the corresponding middle and ventral portions of the inner surfaces

of the lateral plates. Had a similar increase of nerve fibers occurred in the dorsal plate and in the dorsal portion of the lateral plates, the central canal of the medulla might have been completely obliterated. On the contrary, the dorsal cavity and the roof plate have been slightly expanded, apparently through an increased internal pressure from the embryonic cerebro-spinal fluid. One evidence that increased pressure may exist was found in the marked decrease in the size of the central canal of the cord and of the ventral part of the canal in the medulla region.

A second evidence is found in the changes that the walls of the fourth ventricle are undergoing preparatory to becoming a functional organ for the production of cerebro-spinal fluid. In connection with the expansion of the roof plate it will be seen that the dorsal mesenchyme has become decidedly vascular (fig. 46, *B.V.*), making easy an infiltration process into the ventricle. Also ependymal cells, surrounding the ventricle, are taking on form and are probably assuming a secretory function, if they have not acquired one previously. From the recent work of Dandy and Blickfan based on the action of certain drugs and on the chemistry of cerebro-spinal fluid, it is evidence that cerebro-spinal fluid must be a product of secretion as well as of infiltration and diffusion. It is a well-established physiological fact that certain secretory cells, as for example the salivary glands, may assume a definite polarity, and produce a secretion against a very strong pressure, even stronger than that of the blood. Some investigators hold that lumina in glands are the result of pressure from secretion.

In the light of these facts, it is fair to assume that the marked lateral expansion of the fourth ventricle and its roof plate exhibited in the 26 day series (figs. 50 to 52) are the direct result of internal pressure caused by the marked increase in cerebro-spinal fluid. The general increase in size of the ventricle together with the marked convexity of the roof plate and the concavity of the internal surface of the lateral plates certainly suggest internal pressure from the cerebro-spinal fluid. It should be noted in figures 50 to 52 that this expansion of the

fourth ventricle has produced a considerable secondary splitting in the concrescence of the lateral plates recorded for the previous series. Apparently this fissure did not penetrate so deeply in the region of the auditory vesicles (fig. 51, *Aud.V.*) as it did in front of them (fig. 50) or behind them (fig. 52), which probably indicates that the growth of the auditory vesicles in some way operated against the further splitting of medulla. It is significant that the expansion of the roof plate and the development of its mechanisms for infiltration and secretion occur at a time prior to the entrance of blood vessels into the wall of the brain and cord. The large production of cerebro-spinal fluid at this time is evidence that it serves some nutritive function.

In figure 54 we have a median sagittal section through the brain of a 26 day Petromyzon, representing a stage similar to that of figures 50 to 53. Especial attention is called to the fact *that figure 54 demonstrates conclusively that the pronounced roof expansion displayed in figures 50 to 52 has occurred without the aid of a pontine flexure.* The slight convexity of the floor of the medulla can be attributed to the increase of fibers. Also earlier and later series revealed that Petromyzon possesses no pontine flexure. Attention should be called to the fact that the marked convexity of the cephalic end of the thin roof plate of the fourth ventricle (*C.C.*) in figure 54 gives every appearance of being under internal pressure from cerebro-spinal fluid. This section also makes clear, as Sterzi has previously shown, that the fourth ventricle is formed from the dorsal portion of the embryonic central canal; while the central canal of the cord is formed from the ventral portion.

The following observations were noted in connection with the appearance of the fourth ventricle in Petromyzon, beginning at a stage when the medulla was a solid cord of undifferentiated nuclei in a syncytium of protoplasm: 1) The ependymal nuclei migrated a short distance to either side of the median sagittal plane leaving a narrow strip of granular protoplasm in the center. 2) A median sagittal furrow or seam appeared in the central protoplasm, extending from the roof plate to the floor plate. 3) An isolated cavity appeared at the dorsal and ventral ends

of this furrow. 4) The two cavities became connected, forming a cleft-like canal, designated as the typical embryonic central canal. This canal seems to be formed by a disintegration of the central ends of the ependymal cells, now in the form of a syncytium. 5) A considerable increase in the size of the dorsal portion of the central canal occurred through the upward and outward migration of some of the roof plate nuclei and a disintegration of the inner protoplasm. No similar expansion of the ventral portion of the central canal of the spinal cord took place because its marginal layer became reinforced very early by the addition of nerve fibers. 6) Following the formation of the central canal of the medulla there was an increase of cells and fibers in the lateral walls, but for the space of about six days there was little change in the size of the central canal. 7) Next a very pronounced increase of fibers occurred in the median and ventral portions of the lateral plates, which brought about a complete concrescence of the corresponding inner margins of the lateral plates. 8) A sufficient amount of cerebro-spinal fluid was formed by infiltration and secretion to produce a marked expansion of the fourth ventricle. This expansion pushed apart the dorsal portion of the lateral plates, which had not been thickened by an addition of fibers, and stretched the roof plate to a much greater width. 9) Along with this expansion a secondary splitting of the concrescence noted in (7) took place. This fissure did not penetrate so deeply in the region of the auditory vesicles on account of the mechanical obstacle offered by these vesicles. 10) This expanded roof plate apparently assumed the function of producing cerebro-spinal fluid at a time previous to the entrance of blood vessels into the central nervous system, when its nutritive function would be of importance.

From a review of the main points in the development of the fourth ventricle in Petromyzon we are warranted in concluding: 1) That a well-developed fourth ventricle and tela chorioidea were formed in one of the lowest living vertebrates, Petromyzon, without the aid of a pontine flexure. 2) The best suggestion that has been given for the appearance of such an organ in the medulla rather than elsewhere in the central nervous system is

that the roof plate has been weakened more in the medulla region through a greater migration of neural crest cells. This explanation, however, is not entirely satisfactory; since it was shown for Petromyzon (fig. 32) that the cranial ganglia were well-differentiated while the medulla was still a solid cord of cells. If then this were the determining factor it must have cast its shadow a long way ahead. Also it is apparent when the difference in size is considered between the medulla and the spinal cord that there are relatively no more neural crest cells extruded from the medulla. 3) The roof expansion appeared at the same time in the region of the X ganglion as in the region of the VIII and V, and developed at a uniform rate throughout the medulla. 4) Two factors are evident in the formation of the fourth ventricle; first, an upward and outward migration of the roof plate nuclei followed by a disintegration of the inner protoplasm; second, internal pressure exerted by the rapidly increasing cerebro-spinal fluid, infiltrated and secreted by the roof plate itself.

D. Selachians and Amphibia. As types of these classes I had access to very complete sets of serial sections of Squalus and Amblystoma embryos, and to one transverse series of a 15 mm. Necturus. A careful examination of this material contributed nothing new to the ontogeny of the fourth ventricle. It was possible, however, to confirm in these forms many of the conclusions arrived at in Petromyzon. In both Squalus and Amblystoma the much earlier appearance of the central canal brings about a much earlier and more extensive expansion of the fourth ventricle. The sections shown in figures 72 and 73, while possessing enormous expansions of the roof plate, exhibit less progress in the differentiation of the structure of the medulla than was revealed in a 12 day Petromyzon (fig. 41), in which very little or no expansion had taken place in the roof plate.

In Squalus and Amblystoma the typical embryonic central canal, in the form of a vertical cleft, is a comparatively late production. The canal appears soon after the neural folds coalesce as a horizontal cleft, its presence often being indicated only by a layer of pigment. This cleft becomes elliptical, then more or less circular, and finally changes to a vertical or dorso-

ventral cleft. These changes in the shape of the central canal suggest a migration of cells, rather than a disintegration process or a splitting apart of the walls as a result of pressure from the secretion of cerebro-spinal fluid. The earliest Squalus series showed an elliptical canal with its longest axis horizontal, this changed to circular, and finally to the so-called typical embryonic central canal (dorso-ventral cleft canal). For a time in the medulla of Amblystoma the roof plate is much thicker than the floor plate, a condition which can be attributed to the presence of a number of neural crest cells in the roof plate, easily distinguishable from the other cells by their large size and spherical form. After the neural crest cells had been entirely separated from the roof, the roof and floor plates are found to be about equally thick. The same thinning out of the roof plate occurs in Squalus in part through the giving off of neural crest cells, which in this species are indistinguishable from the other cells of the medulla. It is evident in Amblystoma (figs. 74 and 83, *R.P.*) and also in Squalus that the roof plate of the medulla has become no thinner than the roof plate of the spinal cord through the throwing off of the neural crest cells. There is no evidence that the roof plate in the medulla is rendered any weaker or any more susceptible to expansion than is that of the spinal cord, through the migration of ganglion cells.

Apparently the first expansion of the roof plate in Amblystoma (fig. 74, *R.P.*) and in Squalus is produced as in Petromyzon by an outward and upward migration of the roof plate cells. The later, more pronounced expansion and stretching of the roof plate can also be ascribed, as in Petromyzon, to internal pressure due to a decided increase of cerebro-spinal fluid (see fig. 72 for Squalus and 73 for Necturus). In both species the dorsal and middle portions of the embryonic central canal of the spinal cord are obliterated by an inward growth of the lateral plates through an addition of fibers. Pressure on the cerebro-spinal fluid might be increased from that source. Also a similar effect would be produced through a marked proliferation of fibers in the medial and ventral portions of the lateral plates of the medulla, which brings about a coalescence of the ventral por-

tions of the lateral plates in Squalus (fig. 72, *C.C.C.*) similar to the condition in Petromyzon. In Necturus (fig. 73) and in Amblystoma the roof plates are expanded to such an extent that no fusion of the lateral plates takes place; nevertheless, the thickening of the walls tends to bring about a reduction in the size of the fourth ventricle and consequent pressure on the cerebro-spinal fluid. The probability of a decided increase of internal pressure from the cerebro-spinal fluid resulting from the reduction in caliber of the embryonic central canal of the spinal cord by at least two-thirds, is more evident, if attention is directed to the fact that the length of the central canal of the spinal cord is fully twenty times that of the fourth ventricle. In both forms the above changes took place before the blood vessels had reached the dorsal surface of the roof plate or entered the medulla. A median sagittal section through a Squalus embryo of the same stage as figure 72 shows that no pontine flexure has appeared.

E. Pig embryos. For making observations on the development of the tela chorioidea of the fourth ventricle in mammals the writer had access to a very complete set of frontal sections of pig embryos from a stage of 4 or 5 mm. up to 14 mm. embryos.

The earliest section (fig. 75), which is from a 4 or 5 mm. pig, discloses that the fourth ventricle has only begun to expand. A large part of this expansion could be attributed to an outward and upward migration of the roof plate cells, and a part to internal pressure from cerebro-spinal fluid, of which traces are beginning to appear as a coagulum (*S.C.F.*). It should be noted that the first blood vessels are appearing above the basal portions of the roof plate, while none have at this stage entered the medulla. Also the ependymal cytoplasm is sufficiently granular to suggest a secretory process, and finally the roof plate has begun to expand before any nerve fibers have appeared in the marginal layer.

A transverse section of the medulla of a 6 mm. pig (fig. 76) revealed a considerable expansion of the roof plate without the aid of a pontine flexure. It is apparent that an increase in the amount of cerebro-spinal fluid is the main factor in bringing

about this pronounced expansion of the roof plate. Since the closure of the dorsal portion of the embryonic central canal of the spinal cord occurs much later in embryonic life, no increase in pressure from cerebro-spinal fluid could take place from that source. Also no increase was shown in the number of blood vessels outside the roof plate, and no blood vessels had entered the medulla. Consequently the only means of an increase of cerebro-spinal fluid would be through secretion and a slight infiltration from the blood vessels. This section demonstrates very strikingly, even more so than figure 75, that the roof expansion begins very early in the development of the pig's medulla, as is evident from the fact that nerve fibers have only begun to appear in the marginal layer.

Between the 6 mm. and 7 mm. stages there occurs a marked increase in the blood vessels in the mesenchyme above the roof plate and a few blood vessels are entering the substance of the medulla. There can be no question that the roof plate is now an efficient organ for the production of cerebro-spinal fluid, and there is a noteworthy increase in the amount of coagulum in the cavity (figs. 75, 76, and 77). The first embryonic cerebro-spinal fluid, probably a mere intercellular fluid, showed little or no coagulum, from the method of fixation and staining used. This may indicate a relative increase of the elements formed by secretion. Figure 77 shows that a marked expansion of the roof plate has occurred in the 7 mm. pig. Since the 7.5 and 8 mm. pig embryos have very small pontine flexures, it is evident that this pig embryo has developed a well-expanded roof plate and chorioid plexus without the influence of a pontine flexure.

The roof plate in the 10 mm. pig (fig. 78, *R.Ex.*) and in the 14 mm. pig (figs. 79 and 80, *R.Ex.*) has undergone a decided expansion, especially in a dorsal direction. This final expansion of the roof plate is unquestionably due to the action of the pontine flexure upon a fourth ventricle filled with cerebro-spinal fluid already under moderate pressure. It would have been impossible for a pontine flexure acting alone on an empty fourth ventricle, as would be implied from His' experiments with bending an empty rubber tube slit dorsally, to have brought about

the dorsal expansion of the roof plate exhibited in figures 79 and 80. Apparently this expansion of the roof plate in the pig has been gradual, for absolutely no stretching of the roof plate has occurred, except in one place, namely in its central anterior portion (fig. 79).

A study of these sections has disclosed a direct relationship between the expansion of the roof plate and the amount of visible coagulum in the ventricle. Since coagulum does not appear in sections of the early fourth ventricle, but does appear after the tela chorioidea has attained the function of producing cerebro-spinal fluid (as is indicated by its vascularity and the granular appearance of the cells) it is fair to assume that the non-coagulable cerebro-spinal fluid found in sections of the early embryos is an embryonic cerebro-spinal fluid, which differs in no way from the ordinary intercellular fluid of other tissues. On the other hand, the coagulum seen in sections after the roof plate has reached the stage of a functional chorioid plexus is evidence of a chemical change in the fluid, which, if a product of secretion, is capable of exerting considerable internal pressure and consequent expansion of the roof plate.

It is apparent that the greater expansion of the roof plate in the pig is produced by the same factors as were recorded for Petromyzon, namely, an early migration outward of the roof plate cells followed by an expansion from within due to the formation of cerebro-spinal fluid, plus the action of a conspicuous pontine flexure on a fourth ventricle filled with cerebro-spinal fluid already under moderate pressure.

F. Human embryos. For this study an 8 and a 15 mm. transverse series and a 23 mm. frontal series were available. These embryos were too far advanced to show the earliest stages of the roof expansion of the fourth ventricle. If, however, the extreme posterior end of the roof plate of the fourth ventricle is examined in the 23 mm. embryo (figs. 26 and 27, *R.Ex.*), in the 15 mm. embryo (figs. 28 and 29, *R.Ex.*), and in the 8 mm. embryo (fig. 31, *R.Ex.*), which represents a region of the medulla little affected by the pontine flexure, it is apparent that the roof expansion was caused by identically the same factors as was

recorded for the pig embryos. In view of the facts, this explanation of the formation of the roof expansion seems more tenable to the writer than to attribute all, as His has done, to the action of a pontine flexure. For beyond question, a considerable expansion of the roof plate in the human medulla takes place before the pontine flexure appears.

2. Description of three roof plate expansions of the spinal cord in the 20 cm. Polistotrema series

An interesting variation (abnormality) was found in the spinal cord of a single specimen of Polistotrema (Bdellostoma), otherwise unusual. Certain structures appeared in the roof of the cord that were very similar to the tela chorioidea of the fourth ventricle, and will be described because of the light that they may throw on the origin of the tela chorioidea of the fourth ventricle.

What has been designated as the first roof plate expansion of the 20 cm. Polistotrema series is shown in the photographs of models 1 and 2 (figs. 1 and 2, 4 and 5, *R.Ex.*) to be an immense outcropping of the roof plate ependyma. Most unfortunately the anterior portion of this specimen, from which the series through the tail was taken, has been lost, so that it is impossible to state how much further cephalad this expansion of the roof plate extended, or whether there were other outcroppings of the roof plate in front of it as there are behind. It appears in the first model (fig. 2, *R.Ex.*) and in the first transverse section (fig. 10, *R.P.Ex.*) as a median mass, covering about one-half of the dorsal surface of the spinal cord; it then shifts gradually over to the right side (figs. 2 and 11); then gradually attains a median position. In this position it continues as far as the middle of model 2 (fig. 5), covering a large part of the dorsal surface of the spinal cord.

For the most part the roof plate expansion contains a cavity of considerable size, which is shown anteriorly in the cast (fig. 3, *C.C.Ex.*) and in transverse section (figs. 10 to 12) to be in direct communication below with the central canal. Posteriorly

such a connection is wanting (fig. 13). The fact that in various places (fig. 13) there is more or less of a string of ependymal cells between the walls of the central canal and the roof plate expansion suggests that in a more embryonic state an open communication existed between the roof plate cavity and the central canal, which became closed in the region of the posterior end of the roof expansion, and persisted in the anterior end. Another variation to be noted in the cavity of the posterior end of the roof plate expansion is that it contains numerous islands and promontories of ependymal cells and connective tissue, shown as white spaces in figure 6. These probably represent portions of the roof plate that have not been completely excavated to form a continuous cavity.

Transverse sections 11 to 13 show the cavity of this first roof plate expansion to be larger than the fourth ventricle in Polistotrema (figs. 64 to 66), and the whole structure more nearly resembles a typical fourth ventricle than does the fourth ventricle itself in this animal. For the most part the walls of the roof plate consist of true ependymal cells, differing in no way from those surrounding the central canal, except for their shorter peripheral processes. Posteriorly connective tissue takes the place of many of these cells. One of these cells is shown in figure 13A to be sufficiently granular to suggest a secretory function. Everywhere the walls of both the roof plate and the central canal are very vascular, suggesting a modified choroid plexus. Figure 13A will demonstrate the ease with which infiltration and diffusion could take place between the blood vessels of the roof plate and its cavity. In figure 11 a fold of the roof expansion, containing a blood vessel, will be seen extending into the cavity, and about it there is collected a mass of coagulated cerebro-spinal fluid (*S.C.F.*).

Of still greater interest are the two posterior outcroppings of the roof plates, designated as roof plate expansions 2 and 3 (figs. 4 and 5, *R.Ex. 2 and 3*), Since these two roof plate expansions are considerably smaller than the first, they can be compared directly with the roof plate of the rhombencephalon of any embryo.

The so-called second roof plate expansion appears in model 2 (figs. 4 and 5, *R.Ex. 2*) immediately behind the first. This is a much smaller outcropping of the roof plate. It contains a cavity (figs. 16 and 17, *C.C.Ex.*), which spreads out a little laterally and caudally, and communicates below with the central canal. This cavity is filled with a fine fibrillar feltwork that stains deeply with orange G, and which for the most part is coagulated cerebro-spinal fluid. The ependeymal walls of both the roof expansion and the central canal are sufficiently vascular to suggest that we have here as in the previous roof expansion, a modified chorioid plexus which is producing cerebro-spinal fluid.

In model 2 (figs. 4 and 5, *R.Ex. 3*) the third outcropping of the roof plate is some little distance behind the second, about equaling it in size. This roof expansion has not been figured in transverse section, but from an examination of a graphic reconstruction of the central canal, three small isolated cavities were seen extending in a cephalo-caudal direction. The middle cavity was found to be in communication below with the central canal. It held cerebro-spinal fluid, and its walls apparently functioned in the production of the same.

It is evident that these three expansions of the roof plate are independent of one another. The arrangement of the three small isolated cavities in the third expansion seems to be an embryonic condition, and suggests that the larger cavities may have been produced by the union of several smaller roof expansions. It may be supposed that these expansions were formed by the multiplication of roof plate cells, which were pushed up in solid masses, in which vacuolization and confluence of adjacent cavities produced the larger cavities seen in the adult.

It is of interest to note that in this individual a posterior sinus (fig. 20, *S.T.*), probably representing the sinus terminalis of a normal individual, was isolated by the complete occlusion of the central canal by ependymal tissue. The ependyma surrounding this cavity is very vascular, and the cavity is distended with cerebro-spinal fluid. This sinus is much larger than the fourth ventricle in a normal individual.

When the roof expansions in the spinal cord of this series of Polistotrema are compared with the fourth ventricle of the higher vertebrate embryos, it is evident that the similarity is only superficial, for the later stages in the medulla oblongata (action of pontine flexure on a thin-roofed neural tube full of cerebro-spinal fluid under moderate pressure), are dependent on factors which are not present in the spinal cord of Polistotrema. The extreme caudal end of the roof plate of the fourth ventricle in the higher vertebrates, however, has not been affected by these later factors, and presents a condition where comparison is made possible. From a comparison of the roof plate expansion in the 23 mm. human embryo (figs. 26 and 27, *R.Ex.*), in the 15 mm. human embryo (figs. 28 and 29, *R.Ex.*), and in the 8 mm. human embryo (fig. 31, *R.Ex.*) with the second roof plate expansion of the spinal cord in the 20 cm. Polistotrema series (figs. 4 and 5, *R. Ex. 2*, and figs. 15 to 17, *R.P.Ex. 2*) and with the third roof plate expansion of the same series (figs. 4 and 5, *R.Ex. 3*), it is evident that the same main factor is present in all, namely, a migration outward and upward of certain roof plate ependymal cells to form an enlarged dorsal cavity. In each case the purpose of this structure is to form an organ for the production and storing of cerebro-spinal fluid. As soon as these structures assumed the function of infiltrating and secretory organs their walls became further expanded from internal pressure of the cerebro-spinal fluid.

From a review of the early stages of the formation of the roof expansion in man, the pig, Amblystoma, Squalus, and Petromyzon, it is fair to assume that the source and early development of the roof expansion of the medulla are identical to the three similar roof plate expansions, described for the spinal cord of a 20 cm. Polistotrema. It is possible that both had their *phylogenetic anlage* as mutations, the former from some primitive vertebrate, and the latter from a normal Polistotrema, that both were useful and dominant characters, and in case of the medulla, where the animal was allowed to reproduce, this character became preserved for the race.

CAUSES UNDERLYING THE FLATTENING OF THE SPINAL CORD IN CYCLOSTOMES

If our attention is first directed to a transverse section through an adult Polistotrema (figs. 10 and 71) it might be inferred, since there is ample room in the membranous neural canal for a well-rounded spinal cord, that the flattening of the spinal cord might be attributed entirely to internal factors. A glance at a transverse section through a developing Polistotrema spinal cord (fig. 57) suffices to show that there is proportionately much less room within the membranous neural canal, and that a mechanical force in the form of a rapidly growing notochord is at work immediately below the spinal cord.

At the outset it seems advisable to establish arbitrarily a typical state of an embryonic spinal cord, by which a direct comparison of one form can be made with another. The examination of very early stages of the spinal cord in a large number of embryos of Squalus, Amblystoma, the chick, and the pig, in all of which the neural tube is formed by the rolling up of the neural plate, shows that the neural tube passes through three stages: a) A depressed tube with the central canal in the form of a horizontal cleft; b) a cylindrical tube with the canal circular in cross section; and c) a laterally compressed tube with the canal in the form of a vertical cleft (figs. 81 to 86). The existence of this series of changes in Squalus has been shown in a table of developmental stages compiled by Scammon. The third stage may be selected as the typical embryonic spinal cord. This stage is reached at about the time of the first appearance of nerve fibers in the marginal layer.

As a result of a comparison of the typical embryonic stages of the spinal cord in the following transverse sections[3] (fig. 39 for Petromyzon, fig. 55 for Polistotrema, fig. 81 for Squalus, fig. 83 for Amblystoma, fig. 84 for the turtle, fig. 85 for the chick, and fig. 86 for the pig) it is clear that we have all gradations from

[3] It is obvious that this comparison would have no value unless the sections were truly transverse sections. To avoid selecting oblique transverse sections, these figures were always drawn from anterior trunk sections if the embryo showed any flexures.

the nearly cylindrical spinal cords of the Cyclostomes (Petromyzon and Polistotrema) to the very much compressed (flattened laterally) cords of the chick and pig. Were it not for the intermediate stages of Amblystoma and the turtle we might be justified in establishing two distinct types of the embryonic spinal cord: type 1 cylindrical, and type 2 compressed. We could even go further and classify the Cyclostome embryonic cord under type 1 and the Gnathostome cord under type 2.

While the equal expansion of the spinal cord through the addition of nerve cells and fibers to the typical embryonic stage in the pig would tend to produce a cylindrical spinal cord, and in the Cyclostome would tend to produce a depressed cord, the internal structure shows that the origin of the differences between the spinal cord of a Cyclostome and a mammal is not so simple. The neural axis of Petromyzon in an early stage, corresponding to stage (a) above, is decidedly compressed instead of depressed. The problem then is to explain the change from a laterally compressed cord in the early Petromyzon embryos to the gradually depressed, ribbon-like, spinal cord of the adult.

A careful examination of figures 55 to 57, which are taken from practically the same region from three different Polistotrema embryos, shows very clearly that the growing notochord is bringing about the marked flattening (depression) and ventral indenting of the spinal cord. In connection with figure 55 it should be noted that the spinal cord is in the so-called typical embryonic stage, and amid surroundings peculiarly favorable for undergoing a depression from a rapidly growing notochord. It will be seen that the spinal cord is closely enveloped by a meningeal membrane (*P.M.*), approximating a layer of connective tissue, the future membranous neural arch, which is firmly attached below to the growing notochord. Directly above, the mesenchyme is proliferating and migrating toward the center to form a median dorsal cartilage (*M.D.C.*); while there is apparently but little lateral resistance in the way of massing of mesenchyme and the formation of myotomes. In a somewhat later stage (fig. 56) some growth has taken place in the notochord, producing a slight indentation on the spinal cord. In a much

later stage (fig. 57) a median dorsal cartilage has been formed. The much stronger membranous neural arches are firmly attached above to this dorsal cartilage and below to the notochord. The soft plastic spinal cord is thus closely confined by the dorsal cartilage above and less closely by the neural arches laterally. The notochord beneath it has grown very rapidly and its enormous increase in size has brought about a decided flattening (depression) of the spinal cord and indentation of its ventral surface.

It is equally clear in Petromyzon also that the growing notochord is to be looked upon as a direct cause for bringing about the flattening of the spinal cord. The external conditions surrounding the spinal cord are shown in figure 58 to be equally favorable for assisting the notochord in this process, with the possible exception that the membranous neural arch is attached above to a membranous neural spine instead of a cartilage, which, however, may be compensated for by an increased dorsal growth of the myotomes.

The hypothesis that the flattening of the spinal cord in Cyclostomes is largely brought about by the upward growth of the notochord after the manner set forth in the previous paragraph is considerably strengthened by the fact that a certain relationship exists between the size of the notochord and the amount of flattening of the spinal cord.

This is clear in the 20 cm. Polistotrema series from sections of the posterior end of the spinal cord (figs. 19, 21, and 22) and from the photographs of the model of the same region (figs. 7 to 9); the flattening (depression) of the spinal cord becoming less evident as the notochord decreases in size. More striking is a similar relationship shown in the tail region of the 70 mm. Polistotrema (figs. 59 and 60) for the reason that these two sections were only one-fourth of a millimeter apart. The above relationship between the size of the notochord and the depression of the spinal cord can be demonstrated fully as conclusively in the medulla region (see figs. 61 and 62). It should be recorded for these two sections that their structure is the same as that of the spinal cord and that they are less than one-half

a millimeter apart. Also the same relationship could be shown in sections anterior to figure 62, and from similar sections of a Petromyzon larva. A possible objection to applying this argument in the tail region might arise from the fact that the extreme posterior end of the spinal cord is non-nervous, consisting entirely of ependyma and undifferentiated embryonic cells. In reply to this we would invite comparison of figures 21 and 22, where the structure is non-nervous in both cases, and where the effect of the notochord is obvious.

That the spinal cord of the higher vertebrates has not been depressed by pressure from the notochord is due obviously to the fact that the notochord is an embryonic structure, which never attains sufficient size to have any influence on the spinal cord. This is clear from figures 85 and 86. In Cyclostomes, however, the notochord is a very important structure, develops early and grows for a long period of time, and serves as the skeletal axis of the adult. In fishes, Amphibia, and reptiles the growing notochord may have some slight effect in flattening of the adult spinal cord. In Amphioxus the ventral surface of the spinal cord clearly shows the indenting effect of the growing notochord (fig. 70), and in the trunk region where the diameter of the notochord is greatest, the spinal cord is most depressed.

GENERAL CONSIDERATIONS AND SUMMARY

From the foregoing facts the following conclusions seem fully warranted:

1) In the development of the roof plate expansion (tela chorioidea) in the medulla oblongata of most vertebrates three separate stages or epochs of expansion should be recognized: a) A first enlargement of the dorsal portion of the embryonic central canal took place from a migration outward and upward of certain of the roof plate cells, or as was the case in Petromyzon from the migration of the nuclei and probable disintegration of the cytoplasm. b) The second stage in the expansion of the roof plate was the direct result of an increase of pressure from the cerebro-spinal fluid, produced from at least two possible

sources. First in the lower vertebrates, as a consequence of considerable embryonic cerebro-spinal fluid being forced into the fourth ventricle from the closure of the dorsal and central portions of the embryonic central canal in the spinal cord and the ventral portion of the embryonic central canal in the medulla, through the union and fusion of the corresponding portions of the lateral plates. Second, in all vertebrates, through the production of cerebro-spinal fluid by the walls of the fourth ventricle assuming the rôle of infiltration, diffusion and secretion. That the ventral portion of the embryonic central canal of the spinal cord was not expanded by the same cause is explained by the fact that it was reinforced at a very early stage by nerve fibers and supported by a growing notochord. c) A third and final stage in the expansion of the roof plate in the higher vertebrates was brought about by the appearance of a pontine flexure acting on a thin-roofed medulla filled with cerebro-spinal fluid, itself under moderate pressure.

2) It is the opinion of the writer that the most important factors in bringing about the expansion of the roof plate of the medulla are those concerned in the second stage described above. For a considerable period of time these factors apparently work in conjunction with the forces of the first stage in resisting counter ingrowths of the lateral plates which would tend to close up the central canal. Since it was shown that no pontine flexure occured in Petromyzon, it can be concluded that the rather extensive expansion of the roof plate in the medulla of this genus was accomplished solely through the factors entering into the first and second stages. For the second stage it was recorded in most cases, especially well shown in the pig, that the size of the fourth ventricle and the expansion of its roof plate bear a close relationship to the amount of coagulum seen in sections. Since this coagulum did not appear in sections until after the roof plate had attained the function of producing cerebro-spinal fluid, it probably indicates a chemical change in the cerebro-spinal fluid, the products of secretion now being added to the tissue fluids which entered the ventricle by infiltration. As a product of secretion, the cerebro-spinal fluid would be capable of exerting

considerable pressure on the thin and plastic roof plate. The tela chorioidea is differentiated as an organ for the production of cerebro-spinal fluid before blood vessels have entered the central nervous system, at a time when the nutritive function of this fluid is important.

3) The appearance of a third stage in the roof expansion of the medulla, due to a pontine flexure, is of little significance save in the higher vertebrates, where it was held that without cerebro-spinal fluid confined in the ventricle there would be no reason for maintaining that a further expansion of the roof plate would take place from the action of a pontine flexure; more than likely, the roof plate would have been folded up within the ventricle. In His' experiment with the bending upward of a dorsally-slit piece of rubber tubing, the elasticity of the rubber tubing, which forced apart the cut surfaces, would be comparable to the action of the cerebro-spinal fluid under moderate pressure within the ventricle, which factor His has apparently disregarded.

4) In the adult Amphioxus there is nothing which for a certainty could be homologized to the fourth ventricle and its expanded roof plate. Two isolated cavities in the region of the roof plate, which might be taken for the anlage of the fourth ventricle, appear to the writer to be nothing more than vestiges of a much larger embryonic central canal. If Amphioxus possesses no fourth ventricle in the adult we may safely conjecture that more primitive vertebrates had a central nervous system in which there was no distinction between medulla and spinal cord.

5) In an attempt to trace the phylogenetic history of the roof expansion of the fourth ventricle in living vertebrates, the peculiar modification of the fourth ventricle in the adult Polistotrema (Bdellostoma) should be recorded here even though it has been accurately described by Sanders, Holm, Miss Worthington, Sterzi, Cole, and Nicholls. From the adult it is evident that the well-formed fourth ventricle of the embryo has become transformed through a process of centralization to a deep-seated canal, for the most part no larger than the central canal of the spinal cord. Of especial interest is the fact that its anterior and pos-

terior portions has developed into specialized organs for the production of cerebro-spinal fluid. Notwithstanding this specialization, the fourth ventricle is thought to be decidedly inferior to the tela chorioidea of Petromyzon as an organ for the production of cerebro-spinal fluid.

6) In the spinal cord of one individual of Polistotrema there occurred at least three expansions of the roof plate which resemble the roof of the fourth ventricle in other vertebrates. From the fact that these expansions were very vascular and their cells granular it is inferred that they functioned as choroid plexuses for the formation of cerebro-spinal fluid. The writer presents the hypothesis that the fourth ventricle in ancestral vertebrates may have originated as a mutation, similar to this sport plexus in the spinal cord of Polistotrema; that such sport expansions may have occurred at various places such as the diencephalic segment, the roof of the mesencephalon where a choroid plexus still exists in Petromyzon, and in the hind brain and spinal cord. Such mutations, proving to be useful have been preserved in the vertebrate race.

Concerning the flattening of the spinal cord in Cyclostomes

7) A great variation in the shape of the so-called typical embryonic spinal cord is to be recorded. In Petromyzon it was found to be nearly cylindrical, to be moderately compressed in Squalus, Amblystoma, and in the turtle, and decidedly compressed in the chick and the pig.

8) To obtain this typical stage the original compressed spinal cord of Petromyzon must have undergone a marked depression, and the early depressed neural tubes of Squalus, Amblystoma, turtle, chick, and pig must have undergone a decided compression. The main factor causing this depression in the former was thought to be ventral pressure from a growing notochord, and the compression of the latter was attributed to lateral pressure from the growing myotomes.

9) Transverse sections immediately before and during the time that the greatest depression of the spinal cord is taking place

in Polistotrema and Petromyzon show conclusively that the main factor involved is the pronounced growth of the notochord. It was further established that the embryonic spinal cord was not only in a very plastic condition, but that the general environment was decidedly favorable for bringing about a depression of the spinal cord through this agency.

10) The conclusions outlined in (9) were considerably strengthened by the fact that a direct relationship was established, in both the medulla and tail region, between the size of the notochord and the amount of depression exhibited in the spinal cord. This was shown in late embryos in both Polistotrema and Petromyzon, and in adult Polistotrema.

11) That a similar depression did not take place in the higher vertebrates from a growing notochord was explained by the fact that the notochord is relatively a transitory and insignificant structure; while in the Cyclostomes it is not only formed early in embryonic life, but grows rapidly and continuously.

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PLATE 1

EXPLANATION OF FIGURES

1 to 9 represent photographs from models of the extreme posterior end of the spinal cord from a 20 cm. Polistotrema (Bdellostoma), illustrating a series of three extensions of the central canal into roof plate expansions. These models were prepared at a magnification of 100 diameters, and were reduced one-half in photographing. In a few cases where certain outlines were somewhat indistinct in the photographs they were strengthened with pen and ink. The roof plate expansions in these models were painted lighter and appear the same in the photographs. Except at the posterior end of the last model, all of the models of the spinal cord exhibit a marked depression and in ventral views they show a pronounced indentation at the center immediately above the notochord. As indicated by an arrow, the caudal direction in figures 1 to 3 is toward the left; while in figures 4 to 9 it is toward the right.

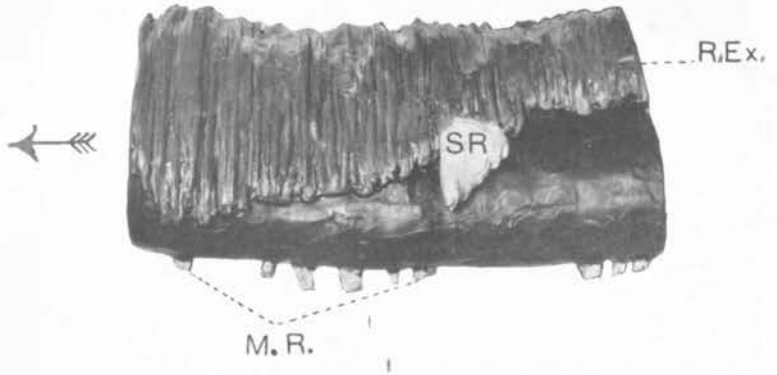
1 The most anterior of the four models seen from the right side. It includes a distance of about two segments. Observe that the roof plate expansion, which covers a large portion of the dorsal surface of the spinal cord, is rather low. It is not known where it begins, or whether there are other outcroppings in front of it as there are behind. It extends caudad some little distance on this model. On the dorsal surface of the cord one dorsal or sensory root is shown, and on the ventral side one ventral root is seen in entirety, being composed of several rootlets. × 50.

2 Dorsal view of the same model shown in figure 1, seen from above. Note that the roof expansion covers a large portion of the spinal cord. At the anterior end it occupies a large central portion, then becomes gradually smaller, and at the same time is confined largely to the left side, after which it gradually increases in size until the posterior end of the model is reached, where it is decidedly wider than at the anterior end, and extends farther over to the right side than to the left. × 50.

3 Ventral view of the cast of the central canal and extension of the same up and out into the roof expansion of the same region of the cord as is shown in figures 1 and 2. It will be seen that the central canal and cavity of the roof plate expansion are connected throughout. In certain places on the right side, shown in white, the roof plate is solid, consisting of ependymal and connective tissue in place of a cavity. For some distance posteriorly on the right side, where the roof expansion is widest, there is no cavity in the roof expansion. The knob-like projections from the right side of the cast of the central canal represent diverticula, and in sections through such a region there would appear to be two central canals. × 50.

ABBREVIATIONS

C.C., central canal or cast of the same
C.C.Ex., central canal extension into roof plate expansion or cast of the same
M.R., motor or ventral spinal nerve root
R.Ex., roof plate expansion
S.R., sensory or dorsal spinal nerve root



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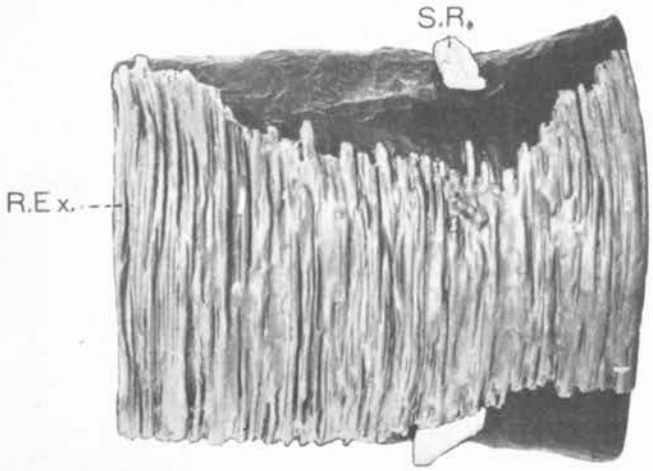

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PLATE 2

EXPLANATION OF FIGURES

4 Lateral view of the left side of the second model of the Polistotrema spinal cord, which should follow figure 1. Observe the continuation of the first roof plate expansion noted in figure 1 and two additional outcroppings of the roof plate (*R. Ex. 2* and *3*). Between these outcroppings the spinal cord is perfectly normal. Three motor and sensory roots are shown in the figure. × 50.

5 Dorsal photograph of the same model as figure 4. The extent and positions of the three roof plate outcroppings previously mentioned in the description of figure 4 are well portrayed here, as are also the sensory roots. × 50.

6 Dorsal view of a cast of the more caudal portion of the central canal and cavity of the first roof plate expansion. This cast differs from the more anterior cast in that there are no connections between the central canal and the cavity of the roof expansion. Evidence from transverse sections favors the view that a connection once existed, which has been cut off in later development. In the two posterior outcroppings of the roof plate there is a connection between the central canal and the cavity of the roof expansion. The light places in the photograph are indicative of places in the roof expansion where it is solid and contains no cavity. They are more numerous and decidedly larger than was shown in the more anterior model (fig. 3). × 50.

Between models 2 and 4 (figs. 4 and 7) comes model 3, the photograph of which has not been included as a figure. It is about equal in length to models 2 and 4, simply connecting the two, without presenting any peculiarities in roof plate expansion, etc.

ABBREVIATIONS

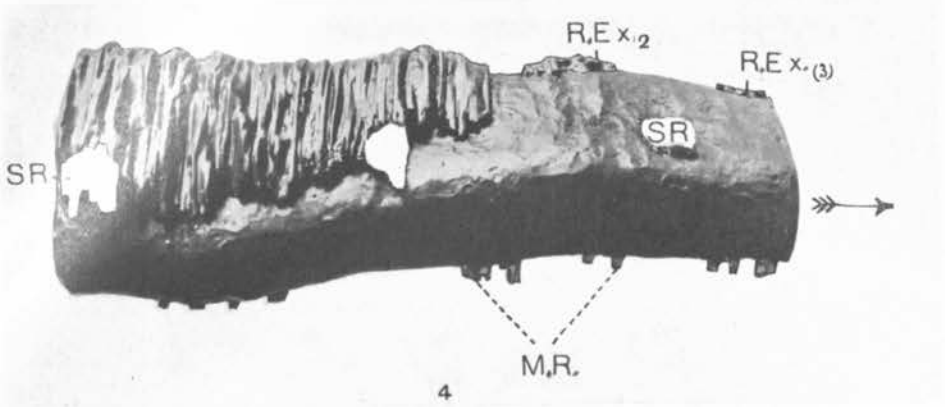
M.R., motor or ventral spinal nerve root

R.Ex., roof plate expansion

R.Ex.(2), second roof plate expansion in the Polistotrema cord

R.Ex.(3), third roof plate expansion in the Polistotrema cord

S.R., sensory or dorsal spinal nerve root



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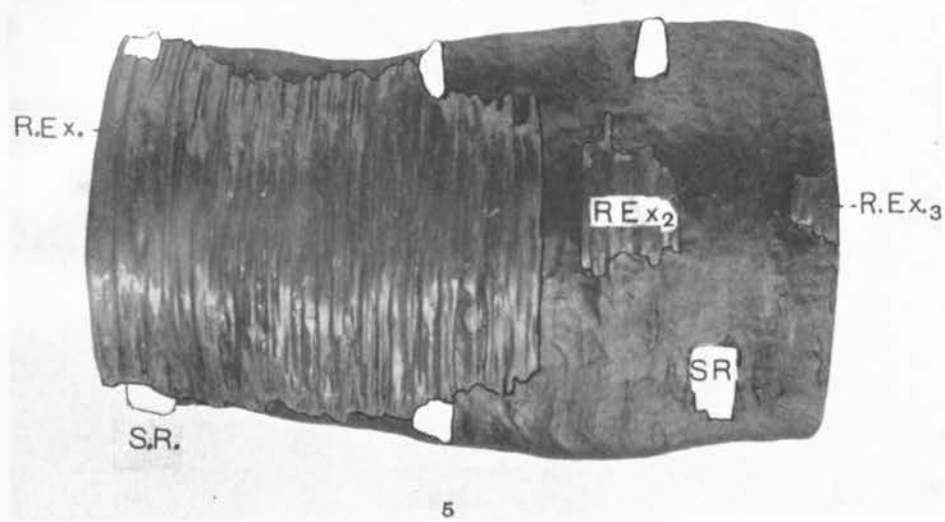

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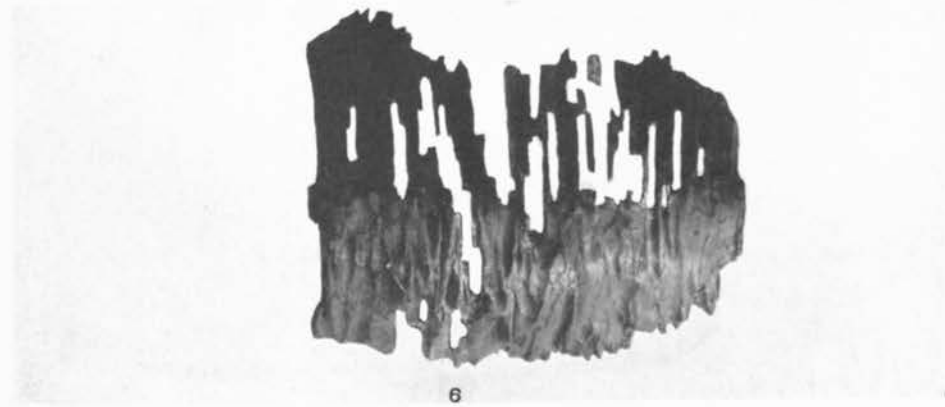

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PLATE 3

EXPLANATION OF FIGURES

7, 8, and 9 represent lateral, dorsal, and ventral photographs of the fourth model, which includes the extreme posterior end of the spinal cord. Note especially the swelling above and below (*S.T.*) caused probably by an abnormal sinus terminalis. From this point caudad two factors occur, which may greatly modify the shape of the spinal cord. First, the notochord gradually decreases in caliber and ends at (*Nc. 1*); second, the spinal cord has not developed a nervous structure, consisting solely of ependyma and round undifferentiated embryonic cells. As a result the spinal cord will be seen to gradually become rounded, and after extending past the notochord it ends in dorsal and ventral processes that become lost in the surrounding connective tissue. The posterior motor roots exhibit a reduction in the number of rootlets, and they approximate each other more closely. The posterior sensory roots become greatly reduced in size, and the last left one has no corresponding roots on the opposite side. × 50.

ABBREVIATIONS

M.R.(1), last motor or ventral spinal nerve root

Nc. (1), posterior end of Polistotrema notochord shown in model

S.R. (1), last sensory or dorsal spinal nerve root

S.T., sinus terminalis

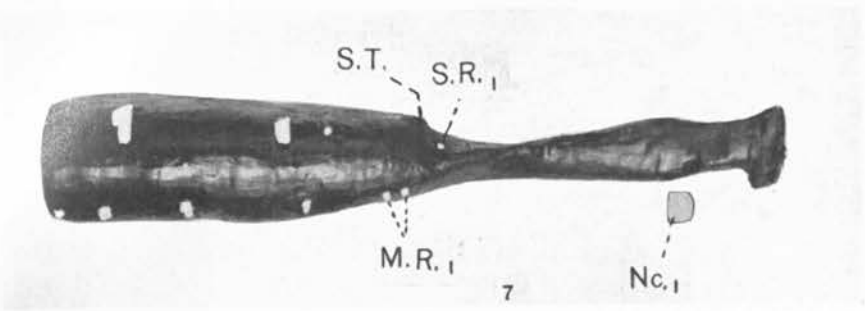
PLATE 4

EXPLANATION OF FIGURES

10 to 23, show 14 transverse sections through the posterior spinal cord region of a 20 cm. Polistotrema (Bdellostoma), the same as was modelled and shown in figures 1 to 9. The outlines were all drawn with the aid of a Leitz-Edinger drawing apparatus, using a magnification of 140 diameters and were reduced one-half in reproduction. They are numbered consecutively from anterior to posterior. Figures 10 to 14 pass through what has been designated as the first roof plate expansion; figures 15 to 17 through the second roof plate expansion; figure 20 through the abnormal sinus terminalis; and figures 21 to 23 at various intervals through the extreme posterior end of the spinal cord, which has failed to develop any nervous structures, and has not to any extent been flattened in its development by the growth of the notochord. The enormous concavity seen on the ventral surface of spinal cord in figures 11 to 14 is to a large extent an artifact due to the preparation of the series. It should be noted that the cavities of the roof plate expansions are full of a fibrillar feltwork, for the most part coagulated cerebro-spinal fluid, that their walls are moderately expanded by it, and their cells are sufficiently granular to suggest a secretary function.

10 is from the most anterior section of this series. The entire neural arch, median dorsal cartilaginous bar, and a portion of the notochord are included in this figure. The so-called first roof plate expansion covers a large area of the central portion of the spinal cord; its cavity is in communication with the central canal. The ependymal walls of both the cavity and central canal are composed of several layers of cells. Motor roots, motor cells, substantia gelatinosa cells, and blood vessels are to be seen in transverse section. × 70.

(*Continued on page 50*)



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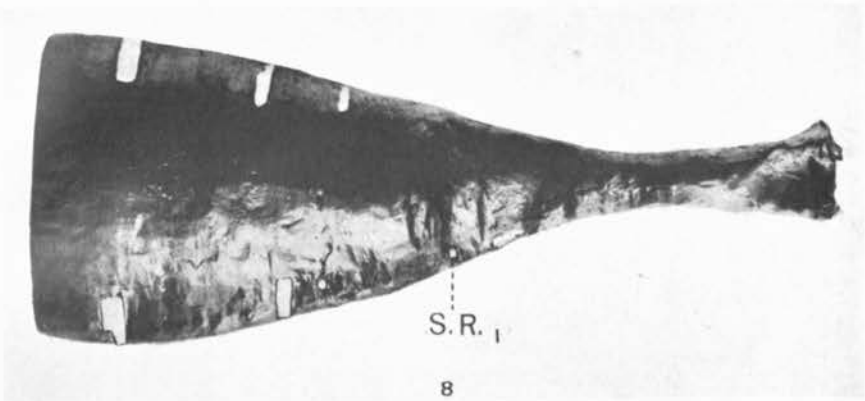

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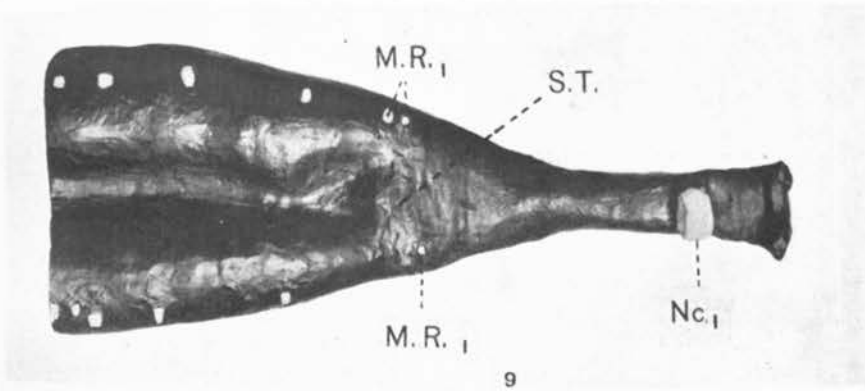

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(*Continued from page 48*)

11 A transverse section taken 840 microns behind figure 10. It would pass through about the center of the first model (figs. 1 and 2). Note that the roof expansion, while containing an enormous cavity, is confined almost entirely to the right side. It is decidedly suggestive of a chorioid plexus forming cerebro-spinal fluid. A blood vessel lies in a fold in its wall and about this fold there is a great mass of coagulum. Throughout the first roof plate expansion, the cavity, and in many places the central canal itself, is larger than either the third or the fourth ventricle. × 70.

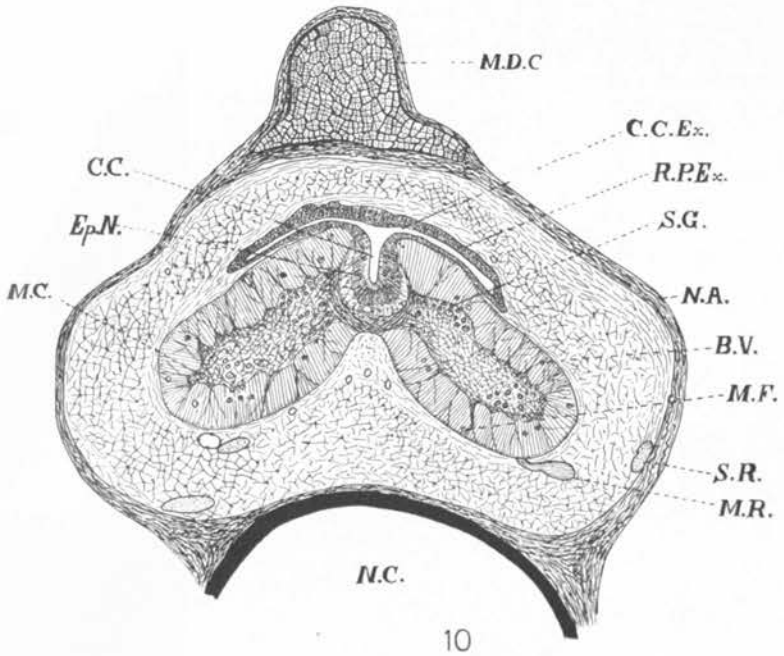
12 From a section 645 microns behind figure 11, and not far from the caudal end of the first model (figs. 1 and 2). At this point the first roof plate expansion and its enclosed cavity attain their greatest width, which cavity is broadly connected with the central canal. × 70.

13 Transverse section 80 microns behind figure 12 and at the very beginning of model 2 (figs. 4 and 5). The dorsal wall of the first roof expansion is very wide and extremely vascular, and the convexity of its walls and the arch of the cavity bear evidence of moderate internal pressure from cerebro-spinal fluid. The wall, while still quite thick, contains a lesser number of ependymal cells, but more connective tissue. Note especially the absence of any direct connection between the cavity of the roof expansion and the central canal. A chain of ependymal cells still connects the two, and may be indicative of a former embryonic connection that has been lost. From this region, caudad, there is no communication between the cavity of the roof expansion and the central canal. That a former connection occurred may be indicated by the fact that at various intervals ependymal cells are scattered between the two. A sensory root can be seen sending its fibers inward toward what is believed to be the substantia gelatinosa. × 70.

13 A, represents a small portion of the first roof plate expansion and its inclosed cavity from a section taken 410 microns behind figure 13. Observe especially the rich blood supply for the dorsal wall and the ease by which diffusion could take place between a blood vessel and the cavity. A large roof plate cell is drawn separately, highly magnified, directly to the left of 13 A. Note the fine granules in cytoplasm, which gives evidence of being secretory. × 125.

ABBREVIATIONS

B.V., blood vessel
C.C., central canal
C.C.Ex., central canal extension into roof plate expansion
Ep.N., layer of ependymal nuclei
M.C., motor or effective cells
M.D.C., median dorsal cartilaginous bar
M.F., Müllerian or giant fiber
M.R., motor or ventral spinal nerve root
N.A., membranous neural arch
N.C., notochord
R.P.Ex., roof plate expansion
S.C.F., cerebro-spinal fluid
S.G., substantia gelatinosa
S.R., sensory or dorsal spinal nerve root



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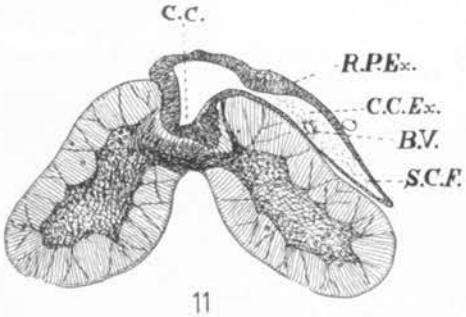

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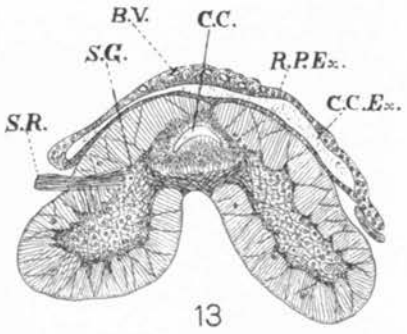

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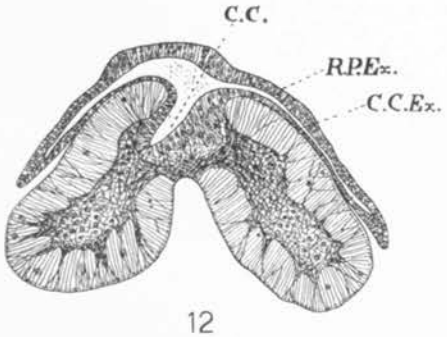

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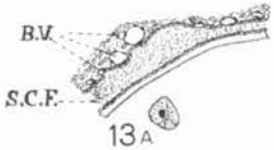


13A

PLATE 5

EXPLANATION OF FIGURES

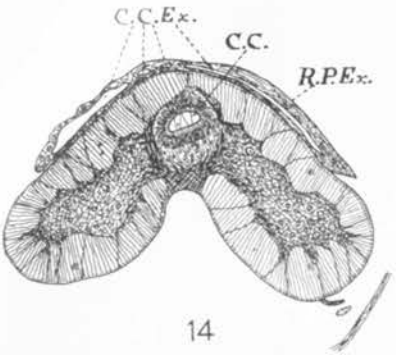
14 From a transverse section taken 19 microns behind figure 13A. It represents a condition found throughout the entire posterior end of the first roof expansion, where a series of cavities are connected with one another. A motor root is seen issuing from the cord. × 70.

15 to 17 Three transverse sections, anterior, middle, and posterior through what has been designated as the second outcropping of the roof plate, which in model 2 (figs. 4 and 5) appears immediately behind the first roof expansion already figured. In figure 15, which is through the anterior end of this roof plate expansion, the outcropping is one of cells only. In figure 16 a distinct cavity containing coagulum is visible, connected below with the central canal. Observe the vascularity of its walls, of the ependyma surrounding the central canal and the diverticula of the same, which doubtless serve as a modified chorioid plexus for producing cerebro-spinal fluid. In figure 17, which is through the posterior end of ths roof expansion, a cavity is still present. Its walls, which are mainly connective tissue, are rich in blood vessels. A few scattered ependymal cells and fibers represent a possible connection with the central canal. A third outcropping of the roof plate has been described, but not figured in transverse section. It is located behind the second expansion, and the fact that no ependymal cells are encountered between the first and the second, and the second and third roof expansions, except immediately surrounding the central canal, favors the view that these separate outcroppings of the roof plate in the adult were never connected in an embryonic state. × 70.

18 and 19 Two sections some distance caudad of figure 17 and some little distance apart. They show the spinal cord to be perfectly normal, but to be gradually tapering down in size. Figure 18 is from that part of the cord represented by model 3. The ependymal area in the center has maintained its original size, the reduction that has occurred in size is to be found in the nervous portion. Not only does the ependymal area broadly divide the spinal cord into two halves, but it has here as elsewhere in a number of places, entirely obliterated the central canal for a space of a few microns. Figure 19 is some distance caudad of figure 18, passing through the sensory nerve roots one segment anterior to the abnormal sinus terminalis (model 4, fig. 9, *S.T.*). The spinal cord will be seen to be fast losing its nervous structure, for no motor cells will be seen in this section or in any further caudad. × 70.

ABBREVIATIONS

B.V., blood vessel
C.C., central canal
C.C.Ex., central canal extension into roof plate expansion or cast of the same
Ep.C., ependymal cells
Ep.N., layer of ependymal nuclei
M.D.C., median dorsal cartilaginous bar
M.R., motor or ventral spinal nerve root
M.V.C., median ventral cartilaginous bar
N.A., membranous neural arch
Nc., notochord
R.P.Ex., roof plate expansion
R.P.Ex. (2), second roof plate expansion
S.G., substantia gelatinosa
S.R., sensory or dorsal spinal nerve root



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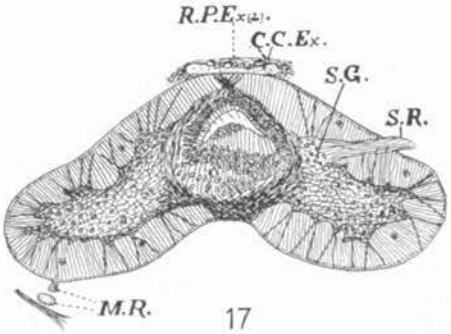

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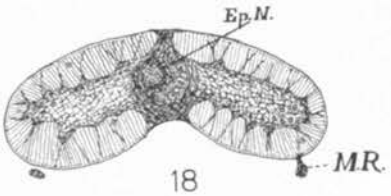

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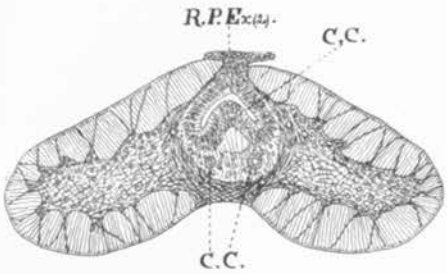

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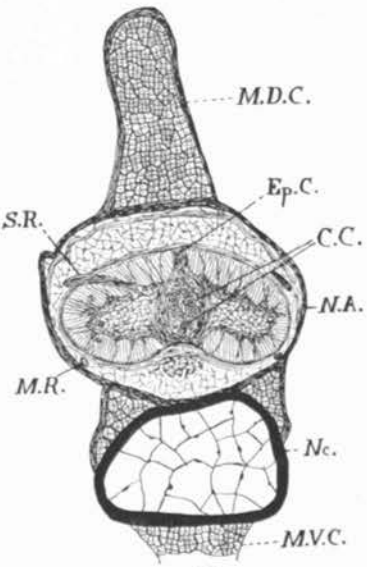

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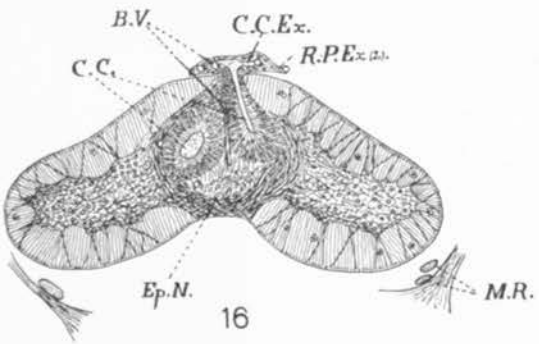

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PLATE 6

EXPLANATION OF FIGURES

20 Through the center of the abnormal sinus terminalis, showing the last pair of sensory roots (not last sensory root) in section. Observe especially the large abnormal sinus together with the rich vascular supply for the tissue immediately surrounding it. Note also the dorsal and ventral swelling caused by this sinus and compare with photograph of the model (figs. 7 and 9). They show every evidence of being expanded by the cerebro-spinal fluid. From this point caudad no nervous structures have developed in the spinal cord. Also from this point caudad the notochord gradually decreases in caliber. × 70.

21 to 23 represent three transverse sections taken through the extreme posterior, non-nervous, end of the spinal cord, which is composed solely of supporting tissue and undifferentiated embryonic cells. Figure 21 is the most cephalic, and passes through the spinal cord a short distance behind the abnormal sinus terminalis (figs. 9 and 20, *S.T.*). The spinal cord is still flattened here, but not indented ventrad, and contains a normal central canal. In figure 22 there has occurred a marked reduction in the size of the notochord. Observe that the spinal cord has not become flattened as it has more anteriorly, where the notochord is massive. With figures 21 and 22 compare figures 59 and 60, which are taken from a similar region of the spinal cord from a 70 mm. Polistotrema embryo. Figure 23 is the last of this series of drawings. It passes through the extreme posterior end of the spinal cord, some 45 microns caudad of the last trace of the notochord. Note the presence of a central canal, and that the cord has separated into several processes, which further on become lost in the surrounding connective tissue. × 70.

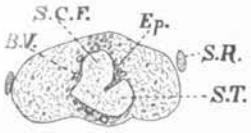
PLATE 7

EXPLANATION OF FIGURES

24 to 31 From transverse frontal sections through the medulla and spinal cord of several human embryos, drawn with the aid of an Edinger-Leitz drawing apparatus, and reduced 3 diameters in reproduction.

24 and 25 From transverse sections through the rhombic brain (frontal through the embryo) of a 23 mm. human embryo; figure 25 passes through the V root and posterior end of the cerebellar rudiments (lateral lobes), while figure 24 is from a more anterior section. The roof expansion (chorioid plexus) is shown as a conspicuous black line in these figures. Everywhere within the boundaries of the roof expansion, the cavity is filled not only with coagulated cerebro-spinal fluid, but with embryonic red corpuscles. Whether these entered through veno-lymphatic openings (C) or are the result of extravasations was not determined. Wherever mesenchyme borders the roof expansion it is very vascular. It is apparent that the roof expansion is under moderate internal pressure. At first glance the roof expansion will show resemblance to the so-called first roof plate expansion of the spinal cord of the 20 cm. Polistotrema, already figured, but its later mode of development was shown to be very different. × 10.

(*Continued on page 56*)



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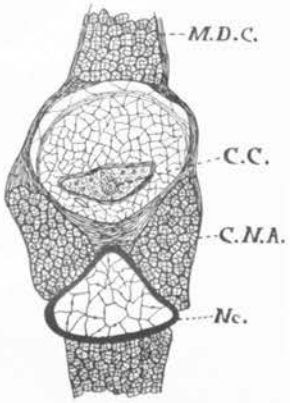

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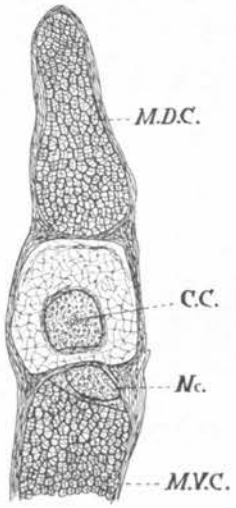

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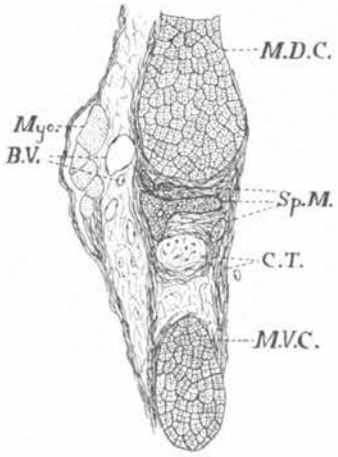


23

ABBREVIATIONS

B.V., blood vessel
C.C., central canal
C.N.A., cartilaginous neural arch
C.T., white fibrous connective tissue
Ep., ependyma
M.D.C., median dorsal cartilaginous bar
M.V.C., median ventral cartilaginous bar
Myo., myotomes
Nc., notochord
S.C.F., cerebro-spinal fluid.
Sp.M., spinal cord
S.R., sensory or dorsal spinal nerve root
S.T., sinus terminalis

(*Continued from page 54*)

26 and 27 Represent two transverse sections through the medulla, passing through the posterior end of the fourth ventricle of the same series from which figures 24 and 25 were drawn. Note especially the expansion of the roof plate and compare with the so-called second roof plate expansion of the 20 cm. Polistotrema spinal cord (fig. 16). It is questionable whether the openings (C) are artifacts or not. As was noted previously, the mesenchyme outside the roof plate is very vascular and the roof plate has the appearnce of being under a moderate degree of internal pressure. × 10.

28 A rather oblique frontal section through the medulla of a 15 mm. human embryo (Inst. of Anat., trans. series, H 23). In this region the contour of the rhombic brain is such that the posterior part of the roof expansion of the fourth ventricle is cut transversely; while the more anterior portion, seen below, appears more or less in frontal section. More anterior sections would show the roof plate to be continuous. The posterior end of the fourth ventricle will admit of direct comparison with the second roof expansion of the 20 cm. Polistotrema spinal cord (fig. 16). Had the fourth ventricle been empty, as was the case of the rubber tubing in His' experiments, there would be absolutely no grounds for believing that the anterior portion of the roof plate would be expanded as it is by the appearance of a pontine flexure. It might on the contrary have been folded up within the ventricle. × 16.6.

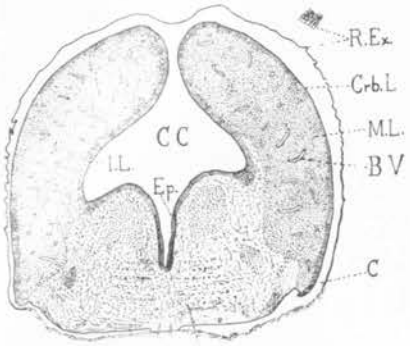
29 A transverse section through the extreme posterior end of the fourth ventricle of the same series as figure 28. There is here a slight roof plate expansion containing no cavity. Compare with figure 15. × 16.6.

30 Transverse section through the thoracic spinal cord, taken from the same series as figure 28. Note that the roof plate consists of ependyma only, while the floor is reinforced by white matter, and even at this late stage if any marked increase in pressure occurred from the cerebro-spinal fluid of this region, an expansion of the roof plate would have been entirely possible. × 16.6.

31 Similar to figure 28, but from an 8 mm. human embryo (Inst. of Anat., series H4). In this plane the posterior end of the fourth ventricle is cut nearly transversely, and is directly comparable with the second roof expansion of the 20 cm. Polistotrema spinal cord (fig. 16). The cavity was full of coagulum, its walls have the appearance of being under moderate internal pressure, and the adjacent mesenchyme is very vascular. × 46.6.

ABBREVIATIONS

B.V., blood vessel
C., an apparent communication between the veno-lymphatics and the fourth ventricle
C.C., central canal
Chon., chondrocranium
C.P., choroid plexus of the fourth ventricle
Crb.L., lateral lobes of the cerebellum
C.T., white fibrous connective tissue
Ep., ependyma
Ex.Ar., external arcuate fibers
I.L., inner or ependymal layer of nuclei
Mar.L., marginal layer
M.L., mantle layer
Ol., inferior olive
R.Ex., roof plate expansion
R.L., rhombic lip
R.P., roof plate of the central nervous system
S.C., semicircular canals
S.R., sensory or dorsal spinal nerve root
T.S., tractus solitarius
VIII.G., auditory ganglion
V.L.S., veno-lymphatic sinus
V.R., trigeminal root
W.R.F., white reticular formation
XI.R., accessory nerve root



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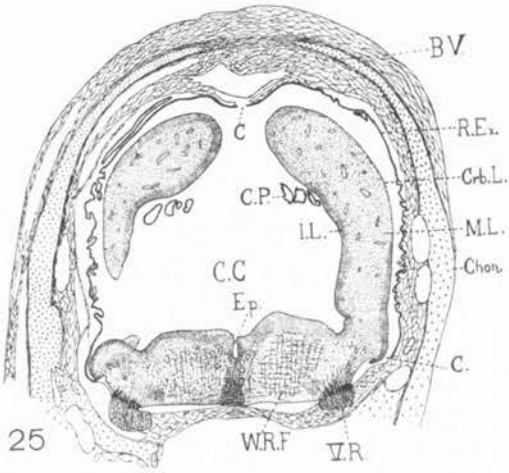

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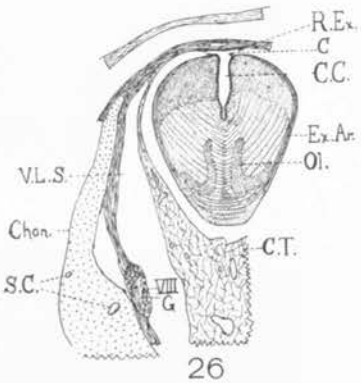

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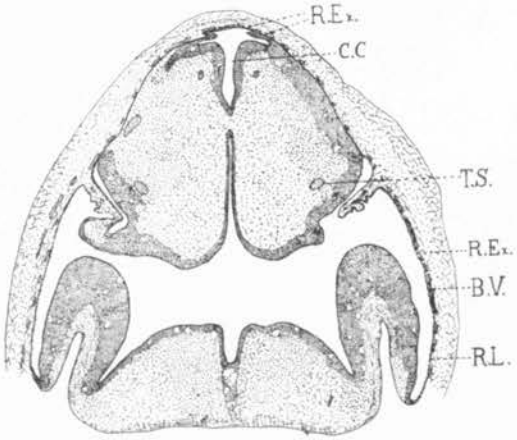

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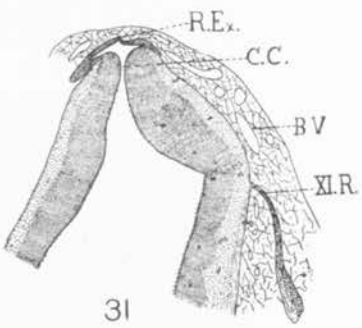

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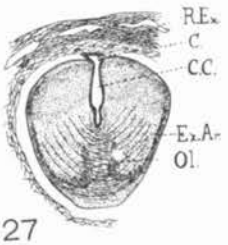

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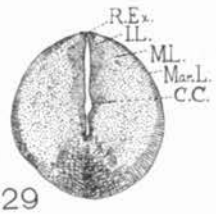

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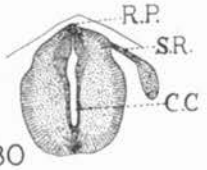

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PLATE 8

EXPLANATION OF FIGURES

32 to 53 A series of transverse sections through the region of the V, VIII, and X ganglia in embryos of Petromyzon of ages varying from 10 to 26 days. It will be seen from these sections that Petromyzon develops an extensive roof expansion without the aid of a pontine flexure, and the cranial and spinal ganglia are well-formed while the central nervous system is a solid cord. All of the figures were drawn with the aid of an Edinger-Leitz drawing apparatus. With figure 54 a magnification of 76.6 diameters was used, while 250 diameters was used for the others. In reproduction they were all reduced one-half.

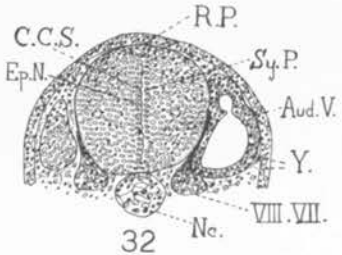
32 Transverse section through the medulla in the region of the auditory vesicle from a 10 day Petromyzon. This is my oldest embryo in which the central nervous system has remained a solid cord. Ordinarily it becomes tubular during the seventh day. This section shows the medulla to consist of a syncytium of protoplasm, consisting of a mass of round nuclei, much yolk, and a few fibers in the marginal layer. The nuclei have migrated a short distance to either side of the median dorso-ventral line. A seam (*C.C.S.*) has appeared here, which marks the position and beginning of the embryonic central canal. The protoplasm bordering the central canal seam is finely granular and may be assuming a secretory function. The acustic ganglia and fibers are shown to be well-differentiated on both sides. × 125.

33 to 35 Three transverse sections passing through the medulla region of another 10 day Petromyzon embryo, in which the central canal has been somewhat retarded in development. These sections pass through the V, VIII, and X ganglia respectively, and with the exception that the central canal furrow or seam (*C.C.S.*) has expanded into small dorsal and ventral cavities (no dorsal cavity has appeared in fig. 35) the general structure of the medulla is about the same as in figure 32. Later these cavities will become the dorsal and ventral expansions of the embryonic central canal of the medulla. The protoplasm in the region of this seam is granular and may be secreting an embryonic cerebrospinal fluid. From these figures it will be seen that the beginning of the central canal occurs at the same time throughout the entire rhombic brain. The anterior portion of the spinal cord, while not figured, contains a central canal furrow in the same stage. × 125.

(*Continued on page 60*)

ABBREVIATIONS

Aud.V., auditory vesicle or otocyst
Br.A., branchial arch
B.V., blood vessel
C.C., central canal
C.C.C., central canal closure, caused by fusion of lateral plates
C.C.S., central canal seam or furrow, in Petromyzon
Ep.N., layer of ependymal nuclei
F.L.P., fused lateral plates of the spinal cord
F.P., floor plate of the central nervous system;
G.C., germinal cell
Mar.L., marginal layer
Mes., mesencephalon
M.L., mantle layer
Myo., myotomes
Nc., notochord
P.B., pineal body
R.P., roof plate of the central nervous system
Sp.M., spinal cord
Sy.P., syncytium of protoplasm
Tel., telencephalon
V.G., Gasserian or semilunar ganglion
VIII.VII., acustico-fascialis ganglion
W.M., white matter
X.G., vagus ganglion (nodosum).
Y., yolk granules



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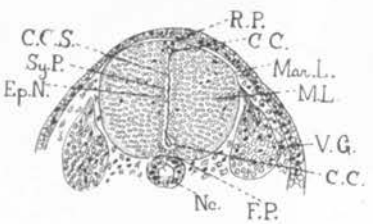

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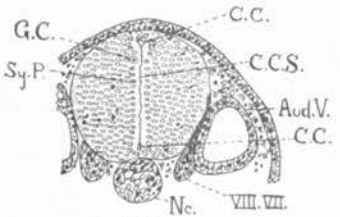

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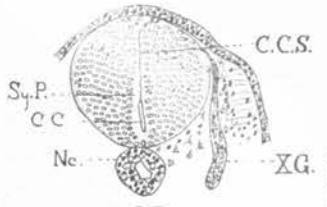

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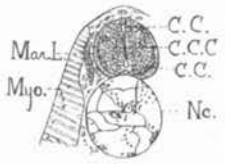

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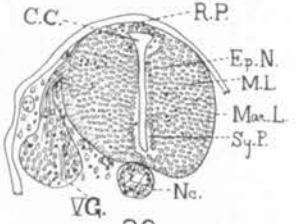

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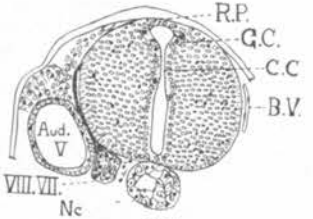

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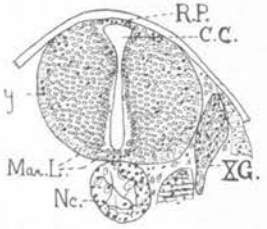

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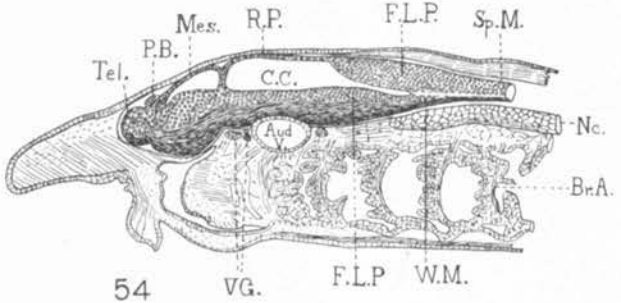

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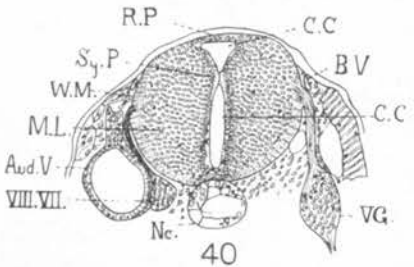

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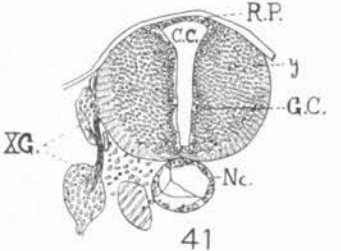

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(*Continued from page 58*)

36 to 38 From a 11 day Petromyzon embryo through the same regions as those shown in figures 33 to 35, and to facilitate comparison were placed directly under them. Considerable progress has occurred everywhere. Note 1) that the central canal seam with its small dorsal and ventral cavities in the 10 mm. series has given place to a typical embryonic central canal, which is much wider at the top and bottom than at the center. The constricted portion of course represents the last place for the protoplasm to give way or to be disintegrated. 2) The floor plate is slightly thicker and less expanded than the roof plate, being reinforced on the outside by white matter and by a rapidly growing notochord below. 3) A few more nerve fibers and nuclei have appeared in the lateral plates. 4) The number of dividing germinal cells has increased while the number of yolk granules remains about the same. 5) The first blood vessels have put in appearance directly outside the meningeal membrane (fig. 37, *B.V.* and nearer the roof plate on the opposite side). × 125.

39 Transverse section through the spinal cord of the same 11 day Petromyzon series as figures 36 to 38, showing the so-called typical embryonic spinal cord. × 125.

40 and 41 Somewhat oblique transverse sections from a 12 day Petromyzon, passing in figure 40, through the V ganglion on one side and the VIII ganglion on the opposite side, and in figure 41 through the X ganglion on one side and a region behind the X ganglion on the opposite side. They can readily be compared with the 11 day series above (figs. 36–38). The lateral plates have apparently increased notably in the number of nerve fibers and nuclei, some of which, however, will have to be attributed to the fact that the sections are cut quite obliquely. Also the numbers of nerve fibers have increased in the floor plate. Throughout, the central canal has increased in width. Of especial interest is a small central mass of protoplasm (*Sy.P.*) in figure 40, which for a space of 50 microns persists as the last remnant of a once solid mass of protoplasm in the center of the medulla. It is obvious at this stage that some factor must have produced sufficient internal pressure to prevent the closing up of the ventricle on account of the rapid increase of cells and fibers in the lateral plates. It is fair to assume that this factor is internal pressure from cerebro-spinal fluid. × 125.

54 of this plate will be described in its proper place, opposite the next plate.

PLATE 9

EXPLANATION OF FIGURES

42 to 44 Three transverse sections through the medulla of an 18 day Petromyzon embryo, passing through the V, VIII, and X ganglia, and for the sake of comparison preserving the same order or arrangement as was used for the earlier embryos. A slight increase in the white matter is to be noted for the lateral and ventral plates over the 12 day series; but little, if any change has taken place in the central canal, unless possibly the central portion has increased slightly in width. Absolutely no further expansion of the roof plate has occurred. Since sections through the medulla of a 14 and 16 day series presented about the same appearance as figures 42 to 44 none were figured. × 125.

45 Transverse section through the cephalic portion of the spinal cord from the same series as figures 42 to 44. Observe especially the beginning of the dorsal closure of the central canal (*C.C.C.*), showing the central canal to consist of dorsal and ventral cavities and a central seam, strongly resembling the stage when it first appeared in the spinal cord. This dorsal closure of the embryonic central canal begins in the anterior portion of the spinal cord much earlier than the corresponding ventral closure of the embryonic central canal in the medulla, occuring in my series of 14 and 15 days. It is obviously caused by the ingrowth of the lateral plates due to the great increase in the number of nerve fibers. × 125.

46 to 48 Three transverse sections through the same region of the medulla of a 20 day Petromyzon as is shown above in figures 42 to 44 for the 18 day embryo. It will be seen that many noticeable changes have taken place, due primarily to a marked increase of nerve fibers in the lateral and ventral plates, and to a slight increase in the number of cells in the lateral plates. The shape of the medulla has become more compressed (flattened out in a dorso-ventral plane). Unquestionably the increase of fibers in the lateral plates has occurred largely in the median and ventral portions. Note the result on the embryonic central canal, which has been completely closed, except for a small dorsal triangular cavity (*C.C.*) the early fourth ventricle. Its roof plate, however, has expanded somewhat. Observe that true ependymal cells are beginning to take on form about the ventricle, and those in the roof plate may soon assume a secretory function, if they are not already active. Also the blood vessels have become more abundant outside of the medulla, especially in the region of the roof plate, which would make infiltration and diffusion through the roof plate into the ventricle easy. × 125.

49 From a transverse section through the anterior portion of the spinal cord. Observe the great increase in fibers in the lateral and ventral plates, and note that the cleft-like embryonic central canal has become entirely closed, but for a small portion, which will remain as the adult central canal. It is obvious that the two portions of the embryonic central canal which persist in the medulla and the spinal cord are the opposite, being the dorsal in the medulla and the ventral in the cord. × 125.

50 to 53 Four transverse sections through the medulla of a 26 day Petromyzon embryo, taken through the V, VIII, and X ganglia, and through a region behind the fourth ventricle where the central canal is passing ventral to assume its characteristic position in the spinal cord. A comparison with the 20 day series above (figs. 46 to 48) will demonstrate a marked increase in the size of the medulla and the fourth ventricle, and a greater expansion and convexity of the

(Continued on page 62)

(*Continued from page 61*)

roof plate, in consequence of which the dorsal tips of the lateral plates are widely separated. There is a marked increase in the number of nerve fibers in the lateral plates, especially in the median and ventral portions. Of prime importance is the great expansion of the fourth ventricle and the roof plate, which apparently in Petromyzon can be explained only from internal factors, the most obvious of which is the mechanical expansion due to an increase in the cerebro-spinal fluid. It will be seen that these forces were sufficiently strong to more than offset the thickening of the lateral plates which would tend to obliterate the dorsal portion of the embryonic central canal as it has the ventral portion. It is apparent that this internal pressure has pushed the lateral wall apart in the dorsal region, where the lateral plates are thinnest and weakest. As was pointed out in the 20 day series the ependymal cells are becoming differentiated and probably have assumed a secretory function. Likewise the increase in the number of blood vessels above the roof plate favors filtration and diffusion into the fourth ventricle. × 125.

54 (See preceding plate.) Median longitudinal section through the head region of a 26 day Petromyzon embryo introduced for a comparison with the transverse sections in figures 50 to 53. Note especially that the marked convexity of the roof plate of the fourth ventricle is suggestive of expansion from an increase of cerebro-spinal fluid. Absolutely no pontine flexure is to be seen, the little convexity that occurs in the floor plate can easily be attributed to an increase in the number of nerve fibers. Observe that the fourth ventricle (*C.C.*) is the remains of the dorsal portion of the original embryonic central canal, while the central canal of the spinal cord is the remains of the ventral portion. The ventral portion of the embryonic central canal of the medulla has been obliterated through the fusion of the ventral portions of the lateral plates. × 38.3.

ABBREVIATIONS

Aud.V., auditory vesicle or otocyst
B.V., blood vessel
C.C., central canal
C.C.C., central canal closure, caused by fusion of lateral plates
Ep., ependyma
Ep.N., layer of ependymal nuclei
G.C., germinal cell
G.M., white matter
M.L., mantle layer
Myo., myotomes
N.C., nerve cell
R.Ex., roof plate expansion
R.P., roof plate of the central nervous system
V.G., Gasserian or semilunar ganglion
VIII.VII., acustico-fascialis ganglion
W.M., white matter
X.G., vagus ganglion (nodosum).

PLATE 10

EXPLANATION OF FIGURES

55 to 62 Taken from various transverse sections through embryonic and larval Polistotrema and Entosphenus (Pacific coast lamprey). Introduced to show the effect of the developing notochord on the spinal cord in Cyclostomes. They were drawn with the aid of an Edinger-Leitz drawing apparatus and reduced one-half in reproduction.

55 Transverse section through the caudal region of a 20 mm. Polistotrema embryo. It will be seen at this stage that the notochord has produced very little visible effect on the spinal cord. Cyclostome embryos of this stage (compare fig. 39 for Petromyzon) present a nearly cylindrical spinal cord; while that of all other vertebrates is more or less elliptical in cross section, the greater

(*Continued on page 64*)

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(*Continued from page 62*)

diameter being dorso-ventral. It should be noted that the spinal cord is enveloped tightly by a meningeal membrane, more or less fused with connective tissue outside that will form the neural arch, which is firmly attached to the notochord below. Immediately above, the mesenchyme is proliferating rapidly and migrating to the center where it will form the median dorsal cartilaginous bar. Little progress has occurred in the formation of the myotomes at the side, and elsewhere there is only loose mesenchyme. × 70.

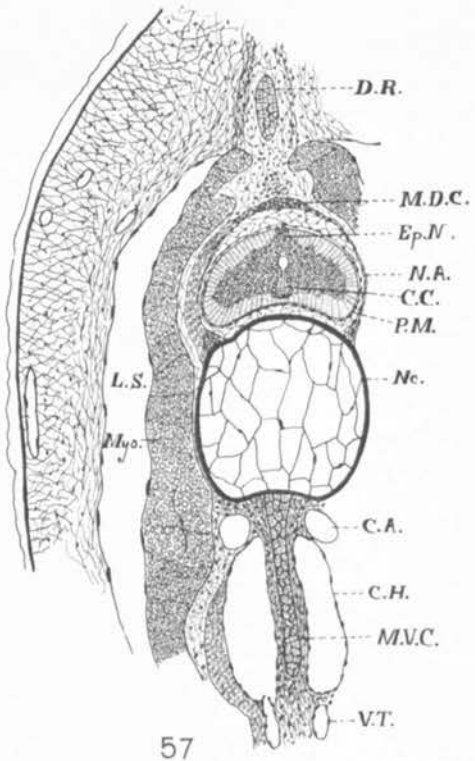
56 Similar transverse section to figure 55, but from a 27 mm. Polistotrema embryo. This slightly later stage shows considerable growth of the notochord and a median indentation on the ventral surface of the spinal cord as the result. Note that the conditions surrounding the development of the notochord previously enumerated under the description of figure 55 are instrumental in assisting the notochord in producing the gradual flattening (depression) of the spinal cord seen in the next figure. × 70.

57 Transverse section of the spinal cord of a 60 mm. Polistotrema embryo from the same region as figure 56. It will be seen that the spinal cord is enclosed in a membranous canal of dense connective tissue, attached below to the notochord and above to the median dorsal cartilaginous bar. Above this there are developing cartilaginous rays surrounded by dense connective tissue. The developing myotomes rest against the neural arches both laterally and dorsally. The notochord has increased greatly in size and, pushing up against the soft spinal cord, produces the depression and ventral indentation of the spinal cord exhibited in this figure. It should be noted that the roof plate is still ependyma and an expansion of the roof plate could take place even in this late stage if the mechanical factors enumerated for the medulla of Petromyzon were operative here. The thickening of the lateral plates has about obliterated the central portion of the embryonic central canal, leaving only the dorsal and ventral portions, in which there is a fibrillar feltwork, probably representing both cerebro-spinal fluid and ependyma cilia. Reissner's fiber is visible in the ventral or permanent central canal. × 70.

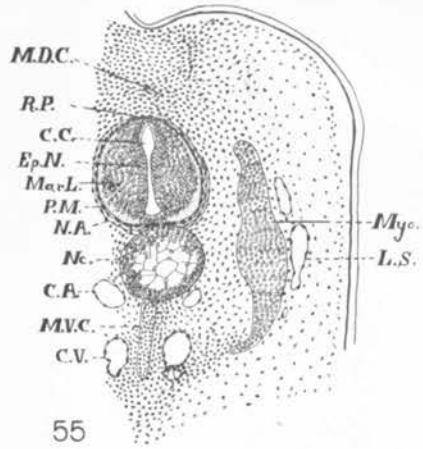
58 Transverse section through the tail region of a 20 mm. Entosphenus larva. It will be observed that the spinal cord is further developed than in the 27 mm. Polistotrema embryo (fig. 56). It is apparent that the same factors are involved in flattening the spinal cord as were enumerated for Polistotrema. The notochord has made fully as much growth and the structures surrounding the spinal cord are the same as in Polistotrema, with the exception that instead of a median dorsal cartilage for the attachment of the membranous neural arch there is a membranous neural spine. To some extent this may reduce the dorsal resistance, but on the other hand it may be compensated for by a greater development of the myotomes above the neural arch. × 125.

ABBREVIATIONS

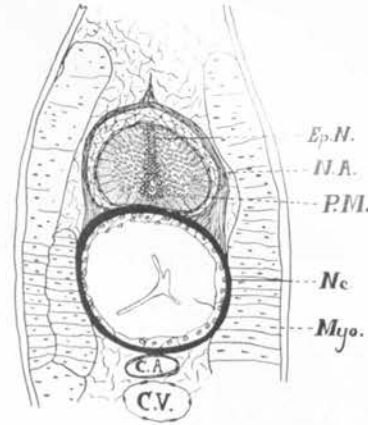
C.A., caudal artery
C.C., central canal
C.H., caudal heart
C.V., caudal vein
D.R., dorsal cartilaginous rays
Ep.N., layer of ependymal nuclei
L.S., lateral veno-lymphatic sinus or anlage of the same
Mar.L., marginal layer
M.D.C., median dorsal cartilaginous bar
M.V.C., median ventral cartilaginous bar
Myo., myotomes
N.A., membranous neural arch
Nc., notochord
P.M., pia mater or meningeal membrane of the younger stages
R.P., roof plate of the central nervous system
Sp.G., spinal ganglion
V.T., ventral veno-lymphatic trunk
W.M., white matter


D.R.
M.D.C.
Ep.N.
N.A.
C.C.
P.M.
Nc.
L.S.
Myo.
C.A.
C.H.
M.V.C.
V.T.

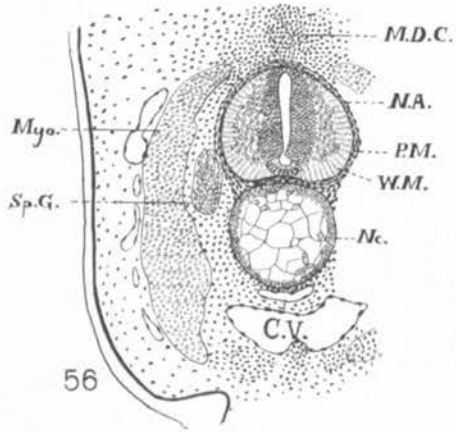
57


M.D.C.
R.P.
C.C.
Ep.N.
Mes.L.
P.M.
N.A.
Nc.
C.A.
M.V.C.
C.V.
Myo.
L.S.

55


Ep.N.
N.A.
P.M.
Nc
Myo.
C.A.
C.V.

58


M.D.C.
N.A.
Myo.
P.M.
W.M.
Sp.G.
Nc.
C.V.

56

PLATE 11

EXPLANATION OF FIGURES

59 and 60 Two transverse sections only 290 microns apart through the extreme posterior end of the spinal cord of a 70 mm. Polistotrema embryo. In the more anterior section (fig. 59) the spinal cord is depressed, contains developing nervous elements, and the notochord is large proportionately. In the posterior section (fig. 60) the diameter of the notochord is much reduced and only supporting elements appear in the spinal cord. As a result no flattening of the spinal cord has taken place in this region. Compare with figures 21 and 22, which are similar sections through an adult Polistotrema. × 70.

61 and 62 Taken from two transverse sections 480 microns apart, through the medulla oblongata of an adult Polistotrema. In both sections no nervous structures have appeared that were not present in the spinal cord. Note as you pass caudad (figs. 62 to 61) that the medulla becomes flattened ventrally and dorsally in direct proportion to the increase in size of the notochord. This relationship can be shown fully as marked in more anterior sections, and in sections taken from a similar region of larval Petromyzon. × 25.

ABBREVIATIONS

Aud.V., auditory vesicle or otocyst
B.V., blood vessel
C.C., central canal
E.N., undifferentiated embryonic nuclei
Ep., ependyma
Ep.N., layer of ependymal nuclei
L.S., lateral veno-lymphatic sinus or anlage of the same
M.D.C., median dorsal cartilaginous bar
M.F., Müllerian or giant fiber
M.L., mantle layer
M.V.C., median ventral cartilaginous bar
Myo., myotomes
N.A., membranous neural arch
N.C., nerve cell
P.M., pia mater or meningeal membrane of the younger stages
P.P., parachordal plate
Sp.G., spinal ganglion
W.M., white matter
X.G. in figure 62 should be *Sp.G.*
X.N., vagus nerve

PLATE 12

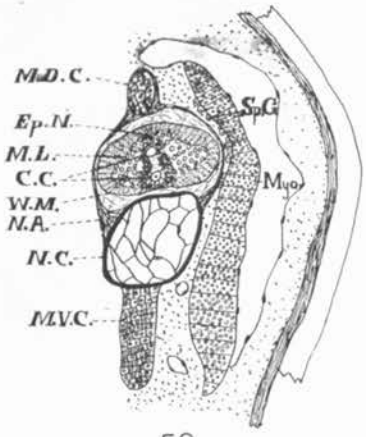
EXPLANATION OF FIGURES

63, represents a diagrammatic reconstruction of the fourth ventricle from an adult Polistotrema series, and the planes from which the transverse sections were drawn for figures 64 to 66 are indicated by lines bearing those figures. Observe how the large fourth ventricle of the embryo has been reduced to a small central canal, having a posterior dilation (P_4V), and how the anterior end breaks up into two or more small longitudinal canals that soon terminate in the sinus mesocœlicus.

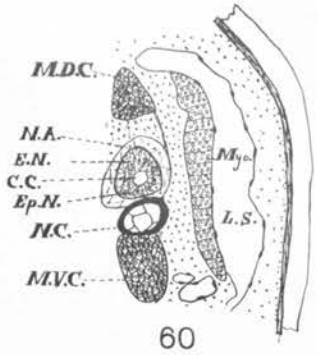
64 and 65, represent transverse sections, taken at different levels of the fourth ventricle of Polistotrema. These sections were drawn with the aid of an Edinger-Leitz drawing apparatus and were reduced one half.

64 From a transverse section through the caudal portion of the mesencephalic lobes (cerebellum of Miss Worthington). Exact plane indicated by line 64 in figure 63. The section passes through the posterior mesocœle or cerebellar ventricle (M''.) and the sinus mesocœlicus (anterior dilation of the fourth ventricle of Miss Worthington), a short distance behind the branching off of the posterior mesocœle. The cavity contains a fibrillar feltwork, which is in part coagulated cerebro-spinal fluid and in part ependymal cilia. The ependyma surrounding the fourth ventricle is rich in blood vessels, which derives its arterial supply from

(*Continued on page 68*)



59



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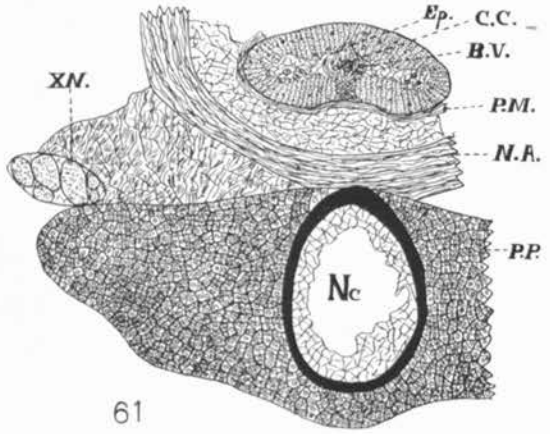
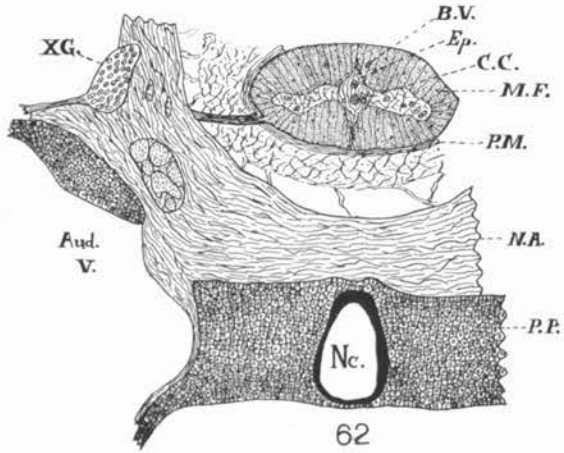


61



62

PLATE 11

(*Continued from page 66*)

two medulla arteries (*M.A.*). Here as elsewhere, the ependyma surrounding the fourth ventricle doubtless functions as a modified chorioid plexus, discharging cerebro-spinal fluid into the fourth ventricle. It will be seen that the cavity of the fourth ventricle is smaller than the peculiarly modified central canal and roof expansion cavity of the Polistotrema spinal cord portrayed in figures 10 to 13. × 25.

65 A more caudal section through the extreme tip of the posterior lobes of the mesencephalon (cerebellum of Miss Worthington), its exact plane being indicated by line 65 in figure 63. It will be seen that the fourth ventricle of the embryo has in this region of the adult become reduced to three small longitudinal canals ($A_4V.$), which are imbedded in a rather large, dense, and vascular ependymal mass. The most dorsal of these canals contains Reissner's fiber. Here again the ependymal walls are probably functional as a modified chorioiod plexus. Very shortly these canals reunite and continue some little distance caudad as a small central canal, no larger than the central canal of the spinal cord. × 25.

66 Transverse section through the posterior end of the medulla of the same series as figure 64. The exact plane of the section is indicated by line 66 in figure 63. It passes through what has been designated as the posterior dilation of the fourth ventricle (P_4V), which is nothing more than a fair-sized centrally located cavity, the remains of a much larger embryonic fourth ventricle, surrounded by a great mass of vascular ependyma. The center of this cavity contains a fibrillar feltwork (*S.C.F.*) composed largely of coagulated cerebro-spinal fluid and some ependymal cilia. Here as more anteriorly we probably have a modified chorioid plexus, the ependymal walls and their blood vessels secreting and filtering cerebro-spinal fluid into the fourth ventricle. × 25.

ABBREVIATIONS

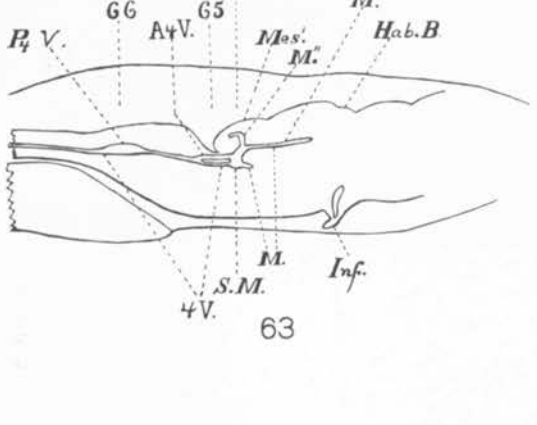
$A_4V.$, anterior fourth ventricle
B.V., blood vessel
Ep., ependyma
Hab. B., habenular body
Inf., infundibulum
M., mesocœle or mesencephalic ventricle
M'., anterior portion of the mesocœle or sub-commissural canal of Nicholls
M''., dorsal portion of the mesocœle or optocoel and posterior portion of the optocoel of Nicholls
M.A., medulla artery
Mes'., posterior lobes of the mesencephalon, cerebellum of Miss Worthington
$P_4V.$, posterior fourth ventricle
S.C.F., cerebro-spinal fluid
S.M., sinus mesocœlicus
V.M.R., motor V root
4V., fourth ventricle
VIII.G., auditory ganglion
X.R., vagus root

PLATE 13

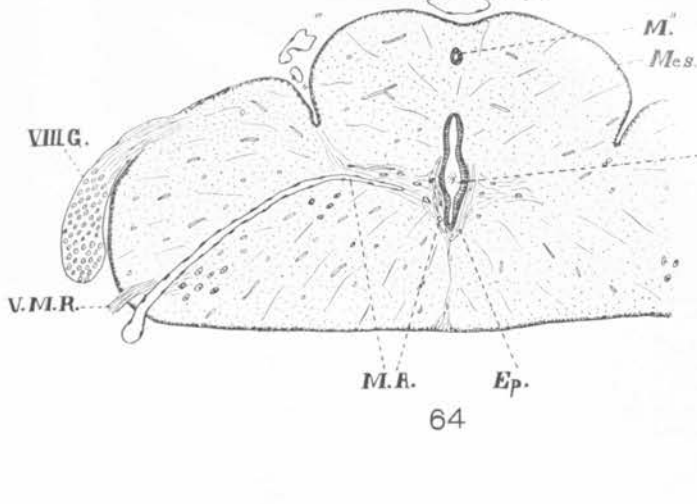
EXPLANATION OF FIGURES

67 to 69 Three transverse sections through the brain region of a 37 mm. Amphioxus. No blood vessels were seen in any of these sections, but the membranous neural canal is surrounded on three sides by enormous veno-lymphatic sinuses, and the structure of the central nervous system is to a considerable extent made up of rather coarse supporting tissue, making infiltration an easy method for nourishing the brain. Drawn with an Edinger-Leitz drawing apparatus and reduced one-half in reproduction.

(*Continued on page 70*)


G4
G6
G5
P_4 V.
A4V.
Mes.'
M.'
Hab.B
M."
M.
Inf.
S.M.
4V.

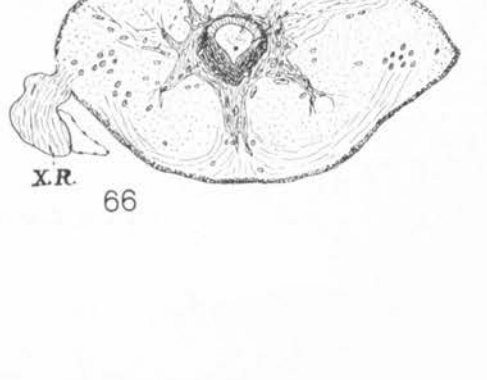
63


M.'
Mes.
VIII.G.
S.M.
V.M.R.
M.R.
Ep.

64


B.V.
Mes.'
A4V.
VIII.G.
B.V.
Ep.

65


S.C.F.
B.V.
Ep.
P_4V.
X.R.

66

(*Continued from page 68*)

The most anterior section, figure 67, passes through the anterior ventricle at its highest point, which is a short distance behind the neuropore. This ventricle has no dorsal dilation suggestive of the fourth ventricle. What dilation occurs, is median and ventral. Cilia-like processes from the border of the cells enter the cavity. If the ependymal cells are not secretory it is possible that the cerebro-spinal fluid of Amphioxus does not differ from the serum of the adjacent veno-lymphatic sinuses. If nerve cells occur in this region they are small, and in ordinary preparations indistinguishable from ependymal cells. × 125.

68 60 microns behind figure 67. The large central canal of the embryonic brain has evidently become reduced in this region to a ventral central canal (*C.C.*) and a small dorsal isolated cavity (*V 2.*). This isolated dorsal cavity can not be compared with the fourth ventricle of higher vertebrates. It is rather to be looked upon as a vestigeal structure, which may aid in the infiltration of lymph from the outer veno-lymphatic sinuses. × 125.

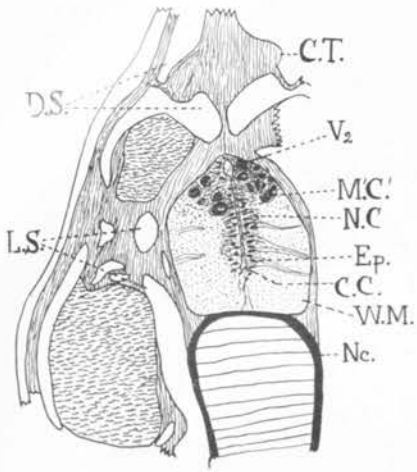
69 Taken from a section 530 microns behind figure 68. It passes through that part of the brain in which there are accumulated a great number of giant cells (*M'.C'.*) in the region of the roof plate. As in figure 68, there is an isolated cavity near the dorsal surface, which was probably a portion of the large embryonic central canal, but which in the adult is separated from the central canal and from the more anterior isolated dorsal cavity by ependyma. It seems best to the writer to regard this and the preceding dorsal cavity as vestigeal structures. × 125.

70 (See next plate.) Transverse section through the anterior spinal cord from the same series as the three previous figures. Observe that the Amphioxus spinal cord is not depressed as is the Cyclostome spinal cord, but is indented ventrally by the notochord. The central canal, which in some places exists as a dorso-ventral cleft, is almost obliterated here by the ingrowth of ependymal tissue. × 125.

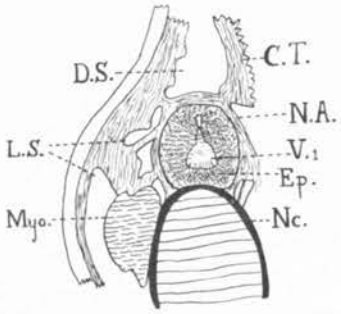
71 Cephalic transverse section through a portion of the spinal cord, meningeal membranes, neural arch, notochord, spinal ganglion,and sensory root of an adult Polistotrema. Observe the depression of the spinal cord, its ventral indentation, the ventral or permanent central canal (*C.C.*), which contains Reissner's fiber, and immediately above, the dorsal portion of the embryonic central canal, which is here more or less filled with ependymal cells and their processes. It will be seen that the gray matter is as much flattened out as is the cord itself, and the ventral horn and motor cells are crowded laterad, while the dorsal horn, substantia gelatinosa (*S.G.*), is apparently median and dorsal. Within the neural arch there is abundant room for a spherical spinal cord. The cord is held in place by the usual meningeal membranes. × 70.

ABBREVIATIONS

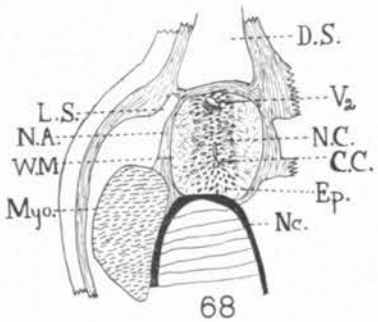
Ar., Arachnoidea
C.C., central canal
C.T., white fibrous connective tissue
D.M., dura mater
D.S., dorsal veno-lymphatic sinus
Ep., ependyma
L.S., lateral veno-lymphatic sinus or anlage of the same
M'.C'., Müllerian or giant cells
M.F., Müllerian or giant fiber
Myo., myotomes
N.A., membranous neural arch
N.C., nerve cell
Nc., notochord
P.M., pia mater or meningeal membrane of the younger stages
S.G., substantia gelatinosa
Sp.G., spinal ganglion
S.R., sensory or dorsal spinal nerve root
Suba.S., subarachnoid cavities
Subd.S., subdural spaces
W.M., white matter
V.1., anterior ventricle Amphioxus
V.2., vestiges of the embryonic central canal in Amphioxus


C.T.
D.S.
V2
M.C.
N.C.
L.S.
Ep.
C.C.
W.M.
Nc.

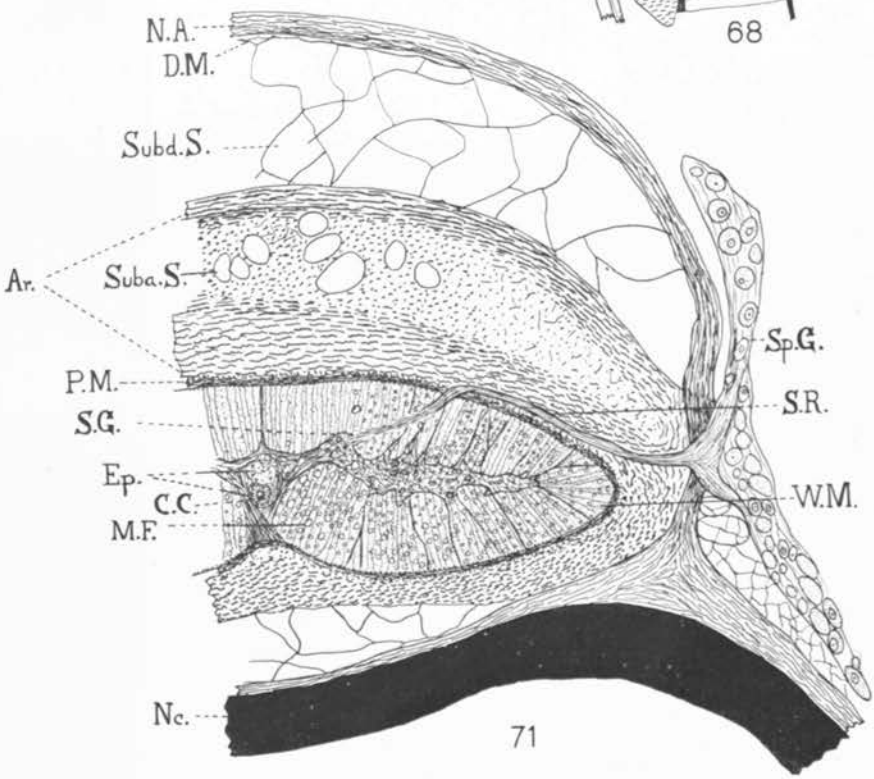
69


D.S.
C.T.
N.A.
L.S.
V.1
Ep.
Myo.
Nc.

67


D.S.
L.S.
V2
N.A.
N.C.
W.M.
C.C.
Ep.
Myo.
Nc.

68


N.A.
D.M.
Subd.S.
Ar.
Suba.S.
Sp.G.
P.M.
S.R.
S.G.
Ep.
C.C.
W.M.
M.F.
Nc.

71

PLATE 14

EXPLANATION OF FIGURES

72 to 80 represent a number of transverse sections through the medulla of shark, amphibian, and pig embryos for the purpose of demonstrating various stages of roof plate expansion.

72 Rather oblique transverse section through the medulla of a 19 mm. Squalus embryo in the region of the VIII ganglion (from series No. 2 of Professor Scammon's collection). Note the well-formed fourth ventricle, and the broadly expanded and very much stretched roof plate. Its collapsed appearance in this section is doubtless due to fixation or preparation. On account of a great proliferation of cells and nerve fibers the lateral plates have fused ventrally, as in Petromyzon, obliterating that part of the embryonic central canal. Attention should be called to the fact that the medulla roof plate in sharks begins to expand much earlier than it does in Petromyzon. This figure shows a well-expanded roof plate, while the cells in the mantle layer are no more differentiated and there are no more nerve fibers in the marginal layer than appear in a 12 day Petromyzon embryo (fig. 40), where there is no fourth ventricle and no expansion of the roof plate. × 70.

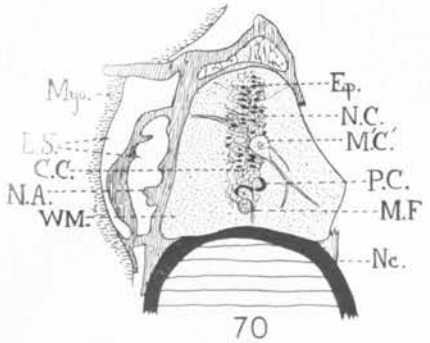
73 Transverse section through the medulla of a 15 mm. Necturus taken through the VIII ganglion. Observe the wide fourth ventricle and the broadly expanded and greatly stretched roof plate, and the coagulated appearance of the cerebro-spinal fluid in the ventricle. Like Squalus, the fourth ventricle begins relatively much earlier than in Petromyzon. It should be noted that no blood vessels have reached the level of the roof plate or entered the medulla; hence the coagulum in the ventricle must be largely a product of secretion. × 39.

74 From a transverse section of a very young Amblystoma embryo in the region of the auditory vesicle (Professor Johnston's series No. 50). Here there has occurred a dorsal and a smaller ventral excavation of the cleft-like central canal. It will be seen that the larger dorsal cavity, the beginning of the fourth ventricle, possesses no thinner roof plate than does the spinal cord (fig. 83). Also in this section (fig. 74) the roof and floor plates are about equally thick. What has taken place dorsally and ventrally throughout the embryonic central canal has been a migration of the cells outward. The fact that in this section of the medulla the roof plate is no thinner than in the section of the spinal cord (fig. 83), taking note that the spinal and cranial ganglia are well-formed, is evidence against the hypothesis, that the greater migration of the neural crest cells of the medulla was the prime cause of the thinning out of the roof plate of the rhombic brain. × 70.

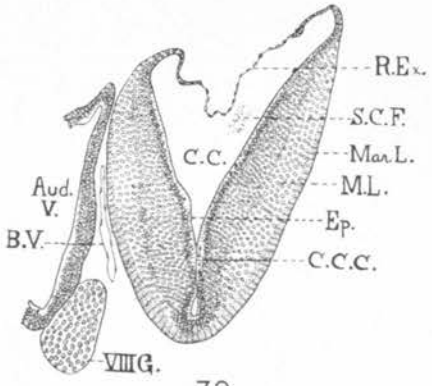
(*Continued on page 74*)

ABBREVIATIONS

Aud.V., auditory vesicle or otocyst
B.V., blood vessel
C.C., central canal or cast of the same
C.C.C., central canal closure, caused by fusion of lateral plates
Ep., ependyma
Ep.N., layer of ependymal nuclei
F.P., floor plate of the central nervous system
G.C., germinal cell
L.S., lateral veno-lymphatic sinus or anlage of the same
Mar.L., marginal layer
M'.C'., Müllerian or giant cells
M.F., Müllerian or giant fiber
M.L., mantle layer
Myo., myotomes
N.A., membranous neural arch
N.C., nerve cell
Nc., notochord
P.C., pigmented or eye cells of Amphioxus
R.Ex., roof plate expansion
R.P., roof plate of the central nervous system
S.C.F., cerebro-spinal fluid
V.G., Gasserian or semilunar ganglion
VIII.G., Auditory ganglion
W.M., white matter
4 V., fourth ventricle



70



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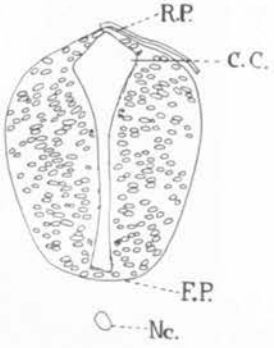

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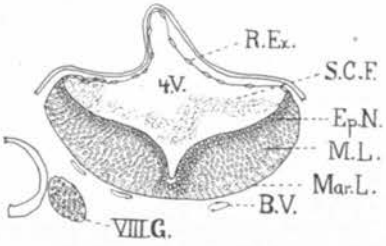
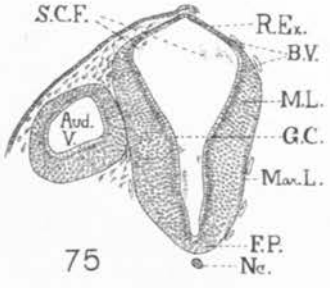


73



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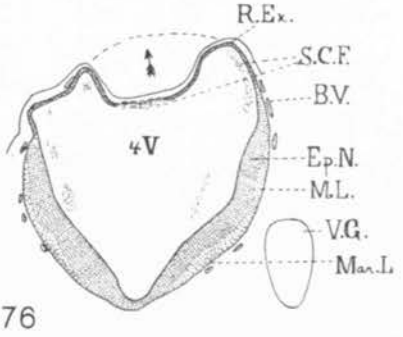


76

(*Continued from page 72*)

75 to 80 represent five transverse sections through the developing fourth ventricle and roof plate expansion in pig embryos from 5 mm. up to 14 mm. With the exception of figure 75, which is from my collection, the remaining figures are from frontal series belonging to the Institute of Anatomy. That there is a direct relationship between the amount of visible coagulum in the form of a fibrillar feltwork and the expansion of the roof plate is evidenced by the fact that this coagulum does not appear in the early embryos before the roof plate has assumed the appearance of an organ capable of the production of cerebro-spinal fluid (as indicated by vascular supply and granular appearance of the cells). It may be inferred that the earliest non-coagulable cerebro-spinal found in the earliest stages is an embryonic fluid which differs in no way from the ordinary intercellular juices, but that the appearance of coagulum at the time when the roof plate has attained the appearance of a functional chorioid plexus is indicative of a chemical change in the fluid, which if a product of secretion is capable of producing a marked increase of internal pressure in the cerebro-spinal fluid and consequent expansion of the roof plate.

75 From a transverse section through the medulla of a 5 mm. (or less) pig embryo, in the region of the auditory vesicle. It will be seen that the peripheral branches of the intersegmental blood vessels have about reached the roof plate, but no blood vessels have entered the medulla. The protoplasm of the inner margin of the ependymal cells is sufficiently granular to suggest a secretory function. The small amount of coagulum in ventricle is probably the result of secretion, but the cerebro-spinal fluid has probably not exerted much internal pressure. × 70.

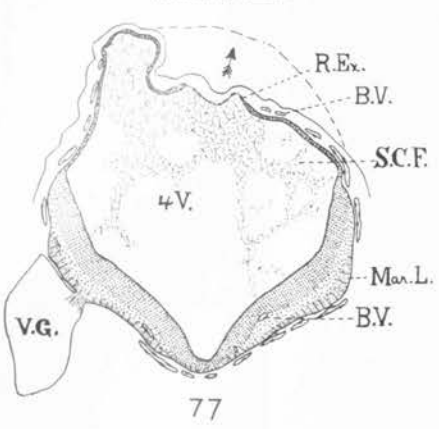
76 Transverse section of a 6 mm. pig medulla through the widest portion of the fourth ventricle, namely, at the level of the V ganglion. This is the only portion of the roof plate to have undergone any stretching of its cells, and this is confined solely to the most centrally located cells. This section shows a considerable increase in the size of the fourth ventricle and expansion of the roof plate, together with some increase in the amount of coagulable cerebro-spinal fluid (*S.C.F.*) and an increase in the number of blood vessels above the roof plate; but no blood vessels have entered the substance of the medulla. At this stage the pontine flexure could not have been a factor in producing the roof expansion. The collapsed appearance of the roof plate at its center is not natural, but rather a result of the preparation of the material. × 39.

PLATE 15

EXPLANATION OF FIGURES

77 Taken from a transverse section through the V ganglion of a 7 mm. pig embryo. Note the increase in the number of blood vessels above the roof plate, which together with the increase in the coagulable cerebro-spinal fluid suggests a functional chorioid plexus. The pontine flexure in this stage is too slight to have any effect on the expansion of the roof plate. It should also be recorded that a few blood vessels have entered the outer surface of the medulla. As in the previous series the sections have suffered a collapse of the roof plate from fixation or later preparation of the material. × 39.

78 From a transverse section of a 10 mm. pig embryo through the region of the V ganglion. The increased vascularity of the mesenchyme above the roof plate together with the enormous amount of coagulated cerebro-spinal fluid (*S.C.F.*) in the ventricle are evidences of the factors which have produced the increased expansion noticed in the roof of the ventricle. Also at this stage the pontine flexure has increased to such an extent that its action on a fourth ventricle full of cerebro-spinal fluid, itself under a moderate pressure, would produce a further expansion of the roof plate. As in the preceding sections the roof plate has suffered a collapse in the preparation of the material. × 39.



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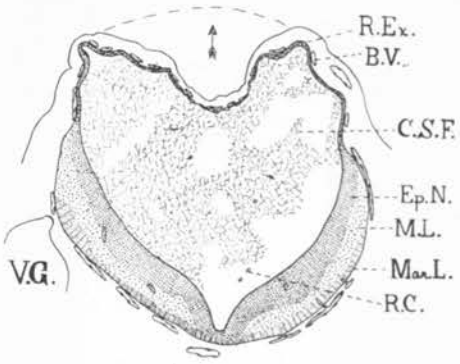

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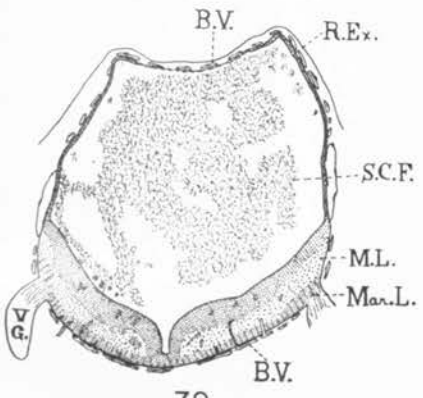

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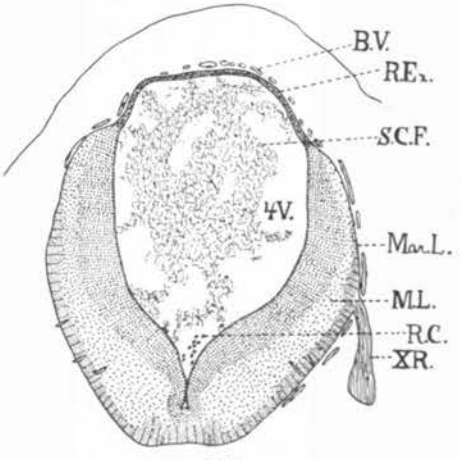

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79 and 80 Two transverse sections through the anterior and posterior ends of the fourth ventricle from a 14 mm. frontal series of a pig. A more advanced stage in the development of the chorioid plexus together with a more pronounced pontine flexure has produced a much larger fourth ventricle and expanded roof plate than is shown in the previous series (fig. 78). The thickening of the lateral walls of the medulla is taking place as it did in Petromyzon and Squalus, but the greater expansion of the ventricle in the pig (fig. 79) has prevented the walls from fusing ventrally. Nevertheless, the thickening of the ventral portion of the lateral plates would increase the pressure of the cerebro-spinal fluid. × 25 and 39.

ABBREVIATIONS

B.V., blood vessel
C.S.F., cerebro-spinal fluid
Ep.N., layer of ependymal nuclei
Mar.L., marginal layer
M.L., mantle layer
R.C., embryonic red corpuscle
R.Ex., roof plate expansion
S.C.F., cerebro-spinal fluid
V.G., Gasserian or semilunar ganglion
4 V., fourth ventricle
X.R., vagus root

PLATE 16

EXPLANATION OF FIGURES

81 to 87 Represent true transverse sections through what has been termed in the text, the typical embryonic spinal cord, from a number of different vertebrates, all of which have developed a tubular nervous system after the neural fold method. They were drawn with the aid of an Edinger-Leitz drawing apparatus and reduced one half in reproduction.

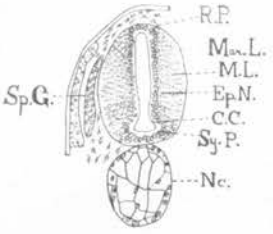
81 From a transverse section through the anterior portion of the spinal cord of a 10 mm. Squalus embryo (Professor Scammon's series No. 16). This so-called typical embryonic spinal cord is decidedly compressed. An earlier stage possessed an elliptical cord with its greatest diameter from side to side. The floor plate is slightly thicker than the roof plate. Each contains a single layer of nuclei. The ventral portion of the cleft-like central canal is expanded into a cavity, which persists as the permanent central canal. A well-formed spinal ganglion is seen to the left. × 125.

82 Transverse section of the spinal cord of a 19 mm. Squalus embryo (from Professor Scammon's series No. 2). Note that the dorsal closure of the lateral plates, due to fiber and cell proliferation, is the same as was figured for Petromyzon. They meet in a seam, leaving dorsal and ventral cavities, of which only the ventral one persists. As in Cyclostomes this method of closure would tend to throw a large part of the embryonic cerebro-spinal fluid into the brain cavities. × 70.

83 and 84 Transverse section through the anterior portion of the spinal cord of an Amblystoma and a turtle embryo. The former (taken from Professor Johnston's series No. 50) is a rather early representative of the so-called typical embryonic stage; while the latter is a rather late representative of this stage. Both cords may be said to be compressed (elliptical, having its greatest diameter dorso-ventral), but only slightly so, when compared with birds and mammals (figs. 85 and 86). As a result, granting an equal proliferation of fibers and cells in the lateral plates, it would be expected that the adult cord in Amblystoma and the turtle would be more depressed, which is found to be the case. × 70 and 125.

85 and 86 From anterior transverse sections of the spinal cord of a 93 hour chick and a 5 mm. pig. Both are good illustrations of the so-called typical embryonic stage, the pig being in a slightly more embryonic state. In these we have the most compressed of all embryonic cords examined, while the adults cord are nearly cylindrical. × 125.

87 Transverse section through the caudal end of the same spinal cord shown in figure 86. Observe spherical appearance which is indicative of an earlier phase in its development. × 125.



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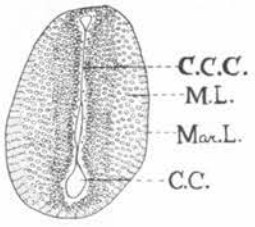

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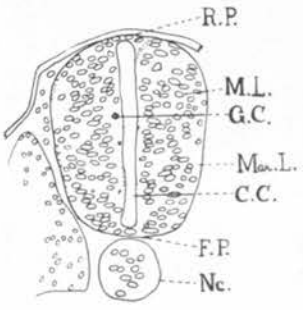

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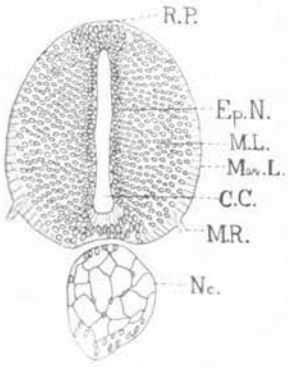

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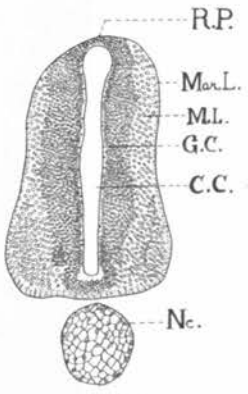

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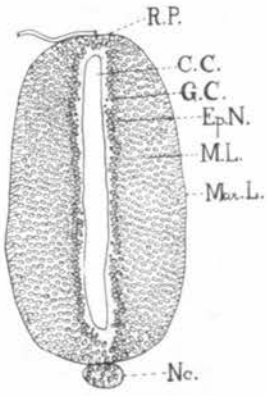

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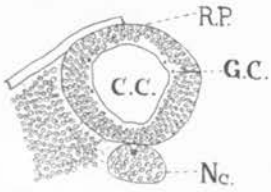

87

ABBREVIATIONS

C.C., central canal
C.C.C., central canal closure, caused by fusion of lateral plates
Ep.N., layer of ependymal nuclei
F.P., floor plate of the central nervous system
G.C., germinal cell
Mar.L., marginal layer
M.L., mantle layer
M.R., motor or ventral spinal nerve root
Nc., notochord
R.P., roof plate of the central nervous system
Sp.G., spinal ganglion
Sy.P., syncytium of protoplasm